SOME ASPECTS OF THE THERMOPHYSIOLOGY OF THE SKINK LEIOLOPISMA ZELANDICA

Richard W. Morris

A thesis

presented for the degree

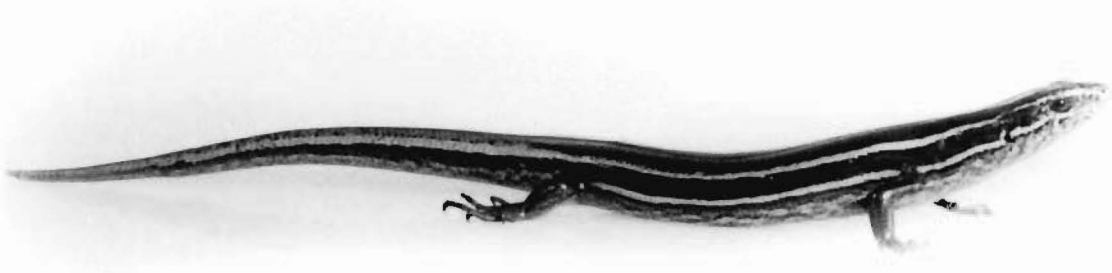
of

Doctor of Philosophy in Zoology

at the

University of Canterbury

September, 1974.



The common or garden skink, Leiolopisma zelandica.

(photo, Terence Williams)

PREFACE

The original aim of this study was to characterise some aspects of the thermophysiology of a terrestrial vertebrate ectotherm which is physiologically adapted to tolerate and to be active over a wide range of body temperatures including low temperatures. The intention was to investigate the physiological performances of a eurythermic lizard over as wide a temperature range as was biologically and technically practicable and at different levels of organisation (organismal, organ and tissue). The small eurythermic skink Leiolopisma zelandica was chosen because of its abundance locally. Preliminary experiments indicated that certain physiological responses of the intact animal could be determined over the range -5° to $40^{\circ}C$.

During the course of this investigation, information about the general biology and thermal relations of L. zelandica was also obtained. In addition, a large number of experiments were conducted at the temperature extremes of this species to explore physiological and behavioural responses to high and low temperatures.

The bulk of the work on the thermophysiology of whole animals and tissues has been written up as two manuscripts in a style suitable for publication (Section 1, Papers A and B).

A second section was erected to contain more thorough accounts of the biology, thermal relations and responses to temperature extremes of L. zelandica (Section 2, Parts A and B).

Because most of the thermophysiological work was

presented in the more concise format of a manuscript, it was necessary to elaborate in appendices on pertinent information regarding equipment and procedures. Data presented graphically in Papers A and B are tabulated in the final appendix.

This approach to thesis presentation has necessitated some duplication of information within the thesis so that the Papers could be relatively autonomous. Since, at the time of thesis submission, Papers A and B have not been sent to editors, a compromise was made between the traditional form of a dissertation and the manuscript style. Consequently, a certain amount of redaction is anticipated.

ACKNOWLEDGMENTS

I would like to thank Professor R.L.C. Pilgrim for his supervision and helpful criticisms. Thanks are also due to Drs V.L. Benzie and H.H. Taylor for critically reading portions of the text. Correspondence with Mr A.H. Whitaker (Ecology Division, D.S.I.R., Lower Hutt, N.Z.) expanded my knowledge of the general ecology of New Zealand lizards.

The assistance of Departmental technicians in the construction of some of the equipment used in this study is appreciated.

I am very grateful to Professor G.A. Knox of the Zoology Department for arranging a Teaching Fellowship which provided an opportunity to gain teaching experience and which assured my family of financial security during this study.

My wife, Cathy, has my sincerest thanks for her assistance and encouragement throughout the study.

SECTION 1

PAPER A — Thermophysiological responses of the eurythermic skink *Leiolopisma zelandica* over the range -5^{o} to $40^{o}C$.

PAPER B — Thermophysiological responses of certain tissues *in vitro* of the eurythermic skink *Leiolopisma zelandica* over a wide temperature range.

CONTENTS

PAPER A Thermophysiological responses of the eurythermic skink Leiolopisma zelandica over the range -5° to 40°C.

LIST OF FIGURES

PAPER A

LIST OF TABLES

PAPER A

PAPER A

THERMOPHYSIOLOGICAL RESPONSES OF THE EURYTHERMIC SKINK *LEIOLOPISMA ZELANDICA* OVER THE RANGE -5° to 40°C

INTRODUCTION

In recent years, thermophysiological responses of predominantly North American and Australian representatives of several lizard families have been reported (for reviews, see Dawson, 1967; Templeton, 1970). These studies have revealed considerable interspecific variation in these physiological responses, some of which corresponds to interspecific differences in the thermal relations of the species studied. Substantial field and laboratory observations on the thermal relations of terrestrial ectotherms have established that lizards and other ectothermic groups - when active under favourable macroclimatic conditions - behaviourally adjust and, in some cases, maintain their body temperature within a narrow range (see Whittow, 1970). With a few notable exceptions (Ruibal and Philibosian, 1970; Veron and Heatwole, 1970), this range tends to be similar for congeneric lizards even if these species occur in different climatic zones (Bogert, 1949; Licht, Dawson, Shoemaker and Main, 1966; Spellerberg, 1972b). On the other hand, sympatric species of different genera may have quite different ranges of temperature when active (Brattstrom, 1965; Cunningham, 1966; Licht *et al.*, 1966). On the basis of these thermophysiological and ecological observations, it appears that an important

factor in the evolution of lizards and other terrestrial ectotherms is their physiological adaptation to behaviourally-adjusted rather than environmentally-imposed body temperatures (see Dawson, 1967; Whittow, 1970).

It is difficult to generalise about the thermal relations of terrestrial vertebrate ectotherms (TVE) in terms of their thermoadjustment behaviour (see Brattstrom, 1965). It appears, however, that obligative heliotherms (true baskers) and obligative non-heliotherms (tropical species particularly) tend to be stenothermic, whereas facultative or opportunistic thermophiles (thigmothermic and limited baskers) are more eurythermic (Morris, 1971), particularly when they occur in temperate regions. Such regions are far from temperate sensu stricto, since they are often characterised by extensive daily and seasonal temperature fluctuations. When compared to stenothermic heliophiles, temperate-dwelling lizards, which are capable of activity over a wide range of body temperatures and throughout the year (i.e. eurytherms), are generally small, viviparous and widely distributed latitudinally and altitudinally. A priori, the most striking differences in thermophysiological adaptations of lizards would be expected between groups with diverse thermal relations such as the relatively stenothermic heliotherms (iguanids, agamids, varanids and teiids) and the more eurythermic groups (scincids, anguids and xantusiids). The present evidence supports this reasoning, although the responses of lizards capable of activity at body temperatures below about 20°C have not been adequately investigated.

The object of this study was to characterise some aspects of the thermophysiological responses of a small

eurythermic TVE living in a temperate region. Oxygen consumption, ventilatory and cardiac rates, and electrocardiograms and electropneumograms (from which detailed analyses could be made) were obtained over the range 0° to 35°C and, when feasible, at -5° and 40°C.

The common New Zealand skink, Leiolopisma zelandica (Gray, 1843), was chosen for this study because of its availability locally. This skink is viviparous as are all but one species of New Zealand lizard. It lives in a wide range of habitats in the South Island: from the sea shore, across the plains and into the mountain areas. In some of these habitats, the substrate temperatures in the winter fall below the freezing point of saurian tissues, whereas in the summer the substrate temperatures can be well above this skink's maximum lethal limit. By burrowing under vegetation and debris, L. zelandica can generally avoid these extreme conditions. But for those lizards living in mountain areas or at high latitudes, the ability to tolerate acute and repetitive supercooling would be important. This capability, however, appears to be universal in reptiles (see Lowe, Lardner and Halpern, 1971) and is widespread amongst other vertebrate and invertebrate groups (for a review, see Smith, 1961). What is unusual about reptiles is that they are the only vertebrates, except for heterothermic bats, which can tolerate several degrees of supercooling without special pretreatment and revive spontaneously after warming (see Lowe et al., 1971).

Although supercooling has been described for many organisms, including reptiles, since about the turn of the century (Kodis, 1898-1902; Bakhmetiev, 1899-1901, both cited by Kalabukhov, 1960), little is known about the physiology of

this phenomenon in vertebrates. Rodionov (1938) reported oxygen consumption in Eremias arguta (Lacertidae) at body temperatures as low as -4.5° to -7.8°C, although this lizard did not survive exposure to temperatures below -5.5°C. Similarly, oxygen consumption was determined for Uta stansburiana (Iguanidae) at temperatures as low as -5°C (Halpern and Lowe, 1968). Cardiac activity has apparently been reported for only one supercooled vertebrate, the bat (Nyctalus leisleri) at -4.3°C (Kalabukhov, 1960). These bats survived several days at body temperatures between -5° and -7°C. Neuromuscular function was determined in vitro for the arctic wood frog (Rana sylvatica) at temperatures down to -5°C (Miller and Dehlinger, 1969). Nerve conduction (in vitro) was reported to occur in R. pipiens to almost -4°C (Jensen, 1972). In this study, oxygen consumption and cardiac activity were determined for supercooled skinks at -5°C.

At the other end of the biokinetic range, summer-acclimatised skinks (L. zelandica) were found to tolerate, for short periods, body temperatures as high as 40°C; this is about seven degrees above its voluntary maximum body temperature. Cardiac and ventilatory responses were determined at this temperature to permit comparisons with other lizards which have been studied.

MATERIALS AND METHODS

Experimental subjects

The common or garden skink, L. zelandica, is probably the most numerous and ubiquitous of the New Zealand reptiles. Most of the skinks used in this study were captured by hand at Kaitorete Spit, about 40 km south of Christchurch, South

Island. Some skinks were also obtained in the Christchurch area. The skinks at the spit inhabited young sand dunes which are sparsely covered with the endemic sand-stabilising sedge, Desmoschoenus spiralis (pingao). The skinks can be collected at the spit on any fine day and at all times of the year as they move rapidly among the pingao when their cover is disturbed. L. zelandica is sympatric at the spit and in other parts of the South Island with the larger and less numerous lizards, L. lineoocellatum and Hoplodactylus pacificus (Gekkonidae).

Although relatively isolated lizard populations nearby were infested with mites, these ectoparasites did not occur on lizards at the spit. The nutritive status of the skinks used in this study, judging by their alertness, celerity and amount of fat storage, was excellent.

After capture, the skinks were toe-clipped for identification. No more than two toes were removed and these were on different feet. Naturally-lost toes (which were not uncommon) were incorporated into the marking scheme. Except when used in experiments, the skinks were kept in an outdoor terrarium (9 m^2) on the roof of the Zoology Building of the University of Canterbury; here they remained acclimatised to the prevailing weather and photoperiod. They were provided with a tray of water and suitable cover, but were not fed in the terrarium. They were, however, given Tenebrio larvae ad libitum in the laboratory, before they were returned to the terrarium after an experiment.

General experimental design

Subadults (from about one to two years of age) and adult

males and post-partem females were used. Individuals ranged between 40-65 mm in snout-vent length and 1.3-4.7 g in weight. To obtain experimental subjects, skinks were collected in the morning before most had begun to bask, and at random from a population of over a hundred kept in the terrarium. From this sample, individuals which had not been used as subjects for at least a week previously were selected.

All temperatures in this study were measured with a Braun "Warburg" thermometer or bead-type thermistors calibrated against this thermometer in a Grant thermostatic bath and are accurate to within 0.1°C. The thermistors were connected to a modified Wheatstone bridge consisting of a series of fixed resistors, helipot and microammeter. This device was designed and constructed in the Zoology Department. To facilitate statistical analysis (see below), the physiological performances were determined at eight temperature stations in the range -5° to 40°C. They occurred at five degree intervals excluding 15° and 25°C. Physiological performances for resting skinks were acutely measured after at least 30 minutes of equilibration (except at 40°C) and over as much of the range as was technically and biologically feasible.

Data were obtained from experiments conducted between 0900-1800 hours and, unless specified otherwise, during the period October 1972 to April 1973 which is coincident with the austral late spring to autumn. In this report, data are presented graphically as semilogarithmic plots against temperature. These data are also tabulated in detail elsewhere (see Morris, 1974, Appendix F).

Oxygen consumption

Oxygen consumption was measured by the direct Warburg manometric method (see Umbreit et al., 1972). Except at 0°C, at least twenty different individuals which were acclimatised to summer conditions were employed at each temperature station in the range 0° to 35°C. The procedures were duplicated during the winter (July-August) over the range -5° to 35°C. Determinations were obtained at only one temperature station for each set of experimental subjects.

Each subject was weighed and placed into a calibrated flask. Quickfit 50 ml flasks had been fitted with centerwells with glass strands across the top to keep the skinks out. Prior to coupling the flasks to their respective manometers and placing them into the glycol-waterbath, 0.5 ml of 20% KOH and a 2 by 1 cm strip of filter paper (Whatman No. 1) folded several times on its long axis were placed into each centerwell to absorb carbon dioxide. The glycol-waterbath was regulated to within 0.1°C except at -5°C where gradual fluctuation of about 0.5°C occurred during any experiment. Up to ten flasks containing skinks, and one which acted as a thermobarometer, were used. After thermoequilibration, three or more determinations were made to obtain a minimum rate for each subject. The duration of the determinations depended on the temperature station and ranged from 3 hours at -5°C to 15 minutes at 35°C. The raw data multiplied by the appropriate flask constants (see Umbreit et al., 1972; Morris, 1974, Appendix B) gave the oxygen consumed in µl under standard conditions (0°C and 760 mm of mercury).

Electroorganography

The recording of spatial and temporal summations of bioelectrical activities of organs and tissues from the surface of a body has been termed electroorganography or EOG (Morris, 1971). With a pair of appropriately-placed electrodes, superimposition of electrical changes can be obtained which are associated with the events of cardiac and ventilatory activities and with general body movements. Such records are called electrocardiograms (ECG), electropneumograms (EPG) and electromyograms (EMG) respectively. EOG techniques facilitated simultaneous determinations of rates and durations of events associated with cardiac and ventilatory performances for resting skinks within the range -5° to $40^{\circ}C$.

From a previous study (Morris, 1971), the orthogonal YY lead, consisting of two platinum electrodes placed in the longitudinal axis, was found to be more satisfactory for unanaesthetised skinks than the leads used by Dawson (1960), Mullen (1967) and others. This lead is easier to apply, relatively free from EMG (i.e. unstressful) and it produces characteristic, analysable waveforms. The active or jugular electrode was implanted subcutaneously about half-way between the forelimb and the ear opening and ventral to the cardiac and thoracic musculature. By autopsy, this electrode was found to lie slightly antero-ventral to the heart. The indifferent or cloacal electrode was inserted with a thermistor into the cloaca. Valentinuzzi, Hoff and Geddes (1969) applied a comparable lead on snakes which skinks resemble in body conformation.

In each experiment, two subjects were taped to balsa wood stretchers which were designed to keep the leads of the electrodes and thermistors in place. The electrodes were

connected push-pull to Tektronix AC Preamplifiers (Type 122), where signals were amplified about 1000 times. These signals were fed either into a sound amplifying system or into Hewlett-Packard DC Preamplifiers (Model 350-2700C) and the separate channels of an H-P Paper Recorder (Model 7712B). The subjects were placed into a temperature cabinet where their body temperatures could be maintained to within 0.1°C of the desired level. EOG experiments were conducted separately with different subjects for the stations from 0° to 35°C, at -5°C and at 40°C. The latter two stations were about a degree (C) from the lethal limits of L. zelandica. For convenience, experiments over the range 0° to 35°C were run from 20° to 35°C, then 0° to 20°C. The subjects were monitored during at least the last five minutes of equilibration at each station to determine that the subjects were inactive. Since L. zelandica tolerated 40°C for only 15-20 minutes, EOG were taken when this body temperature had been reached. After equilibration, EOG were recorded at intervals to obtain the minimum rates at each temperature station. Cardiac and ventilatory rates were determined from records obtained at low paper speeds. Above 20°C, ventilatory rates were often determined by counting sounds which resulted from suitable amplification of high frequency EPG signals. More detailed information regarding the durations of ECG and EPG events was measured from high speed recordings (25 or 50 mm/sec) using Vernier calipers and a stereo microscope in conjunction with a calibrated eyepiece micrometer.

RESULTS

General ecology and behaviour

L. zelandica, as is typical of many scincids, generally keeps itself concealed under vegetation, debris or other suitable cover. However, this skink becomes conspicuously heliothermic during periods of the day when ambient temperatures (particularly those of the substrate) are low, but it remains close to cover. As the temperature of the substrate increases, it moves back into the security of the vegetation where it can make use of filtered insolation and microclimates within the cover. Then, if the insolation is too intense, this small lizard avoids further heating by burrowing beneath its cover. Consequently, L. zelandica tends to be crepuscular in the summer and active during the warmest periods of the day in the winter. It is clear that this skink adjusts its temperature behaviourally, although, once warmed, it may not actively thermoadjust except to avoid overheating (see Heath, 1964). On the basis of these observations, L. zelandica can be described as an opportunistic or facultative thermophile.

The body temperatures of 35 individuals measured in the field ranged from 13.6° to 32.9°C (mean, 25.2°C). These temperatures were obtained under conditions when many of the individuals in the field were observed to be active (see Templeton, 1970). L. zelandica, like other temperate-dwelling scincids which have been studied, is eurythermic (active over a wide range of body temperatures) and has no specific optimum or preferred temperature comparable with that of other, more thermophilic lizards (see Brattstrom, 1965). The fact that L. zelandica occurs as far south as 47°S (Stewart Island) and

on mountains of the South Island, highlights the importance of eurythermy and viviparity as physiological adaptations to the cool habitats and the wide daily and seasonal temperature fluctuations of temperate regions.

Laboratory observations of L. zelandica have indicated that some individuals are capable of voluntary locomotion at body temperatures as low as -1°C, although when placed on their backs they did not always right themselves. In fact, the righting response of this skink is not well developed, since it can be placed on its back at considerably higher temperatures and it will remain in that position for some time. This procedure is, for this skink at least, unsatisfactory for determining the point of immobilisation due to low temperatures, i.e. the critical thermal minimum (see Spellerberg, 1972a). In an effort to determine the supercooling limit (see Lowe, Lardner and Halpern, 1971) individuals were cooled below -5°C. Most subjects spontaneously crystallised just before -6°C was reached. Other leiolopismids studied have similar supercooling limits (Spellerberg, 1972a). Following spontaneous crystallisation, the body temperature rapidly increased to the freezing point of the tissues, about -0.7°C.

When individuals were heated above 35°C, they generally became very restless and attempted to scale the walls of the container in which they were being held. At 40°C, some individuals were conspicuously inactive while others darted at the walls of the container. The former group died without the spasms which precede the critical thermal maxima of most other lizards studied (see Licht, Dawson and Shoemaker, 1966). The hyper-active group lost co-ordination, often falling on to

their backs in their attempts to get out of the container. If these individuals were not rescued immediately when they failed to right themselves, they did not survive. They too died without spasms. For short periods of time, therefore, L. zelandica can tolerate body temperatures about seven degrees above their maximum voluntary body temperature. In terms of this safety margin, this skink resembles the anguid, Gerrhonotus multicarinatus (Cunningham, 1966).

Relation of oxygen consumption to temperature

Rates of oxygen consumption for resting skinks acclimatised to winter conditions were acutely measured between -5° and 35°C. Rates were also determined for summer-acclimatised skinks from 0° to 35°C. The mean values from these two seasons were compared over the range 0° to 35°C using Student's t-test at the 0.05 level of significance for two-tailed distribution. Since the differences were not statistically significant ($P > 0.1$), the data were combined. The means, ranges and two standard errors at each temperature station are plotted semilogarithmically in Fig. 1. The relation of oxygen consumption (expressed as $\mu l\ O_2/g/hr$) to temperature is curvilinear with the slope of a smooth curve drawn between the means, increasing progressively below 30°C and increasing slightly from 30° to 35°C. These changes in slope are reflected in the respective temperature coefficients (Q_{10} values) for the temperature intervals (Table 1).

Relation of ventilatory rate to temperature

Ventilatory rates for resting skinks were acutely measured between 0° and 40°C. When the data are plotted

Fig. 1: Relation of oxygen consumption to body temperature for resting skinks.

Data represent the combined minimum rates (for summer- and winter-acclimatised skinks) which were acutely measured at different temperatures. Differences between means for different seasons were not statistically significant ($P > 0.1$). Range is represented by the length of a vertical line, mean by a horizontal line and two standard errors on each side of the mean (equivalent to about 95% confidence limits) by a shaded rectangle. Number of different individuals is indicated. A smooth curve is drawn through means.

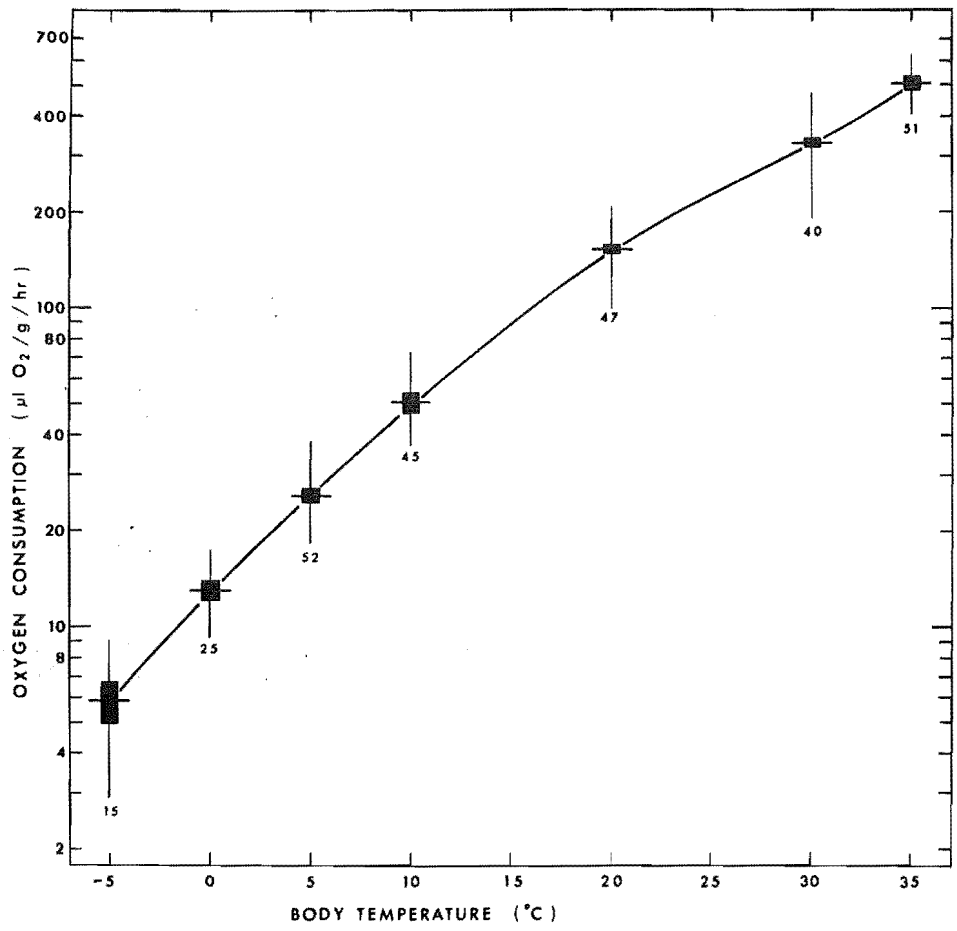

700
400
200
100
80
40
20
10
8
4
2
OXYGEN CONSUMPTION (µl O2/g/hr)
15
25
52
45
47
40
51
-5
0
5
10
15
20
25
30
35
BODY TEMPERATURE (°C)

Table 1: Temperature coefficients (Q_{10} values)* for various physiological rates of resting skinks.

Rate	Temperature intervals (°C)						
	-5 - 0	0-5	5-10	10-20	20-30	30-35	35-40
Oxygen consumption	4.9	4.0	3.8	3.0	2.2	2.4	-
Ventilatory	-	-	2.9	1.7	2.0	1.3	2.6
Cardiac	56	7.9	3.5	2.6	2.0	1.8	1.5

* Q_{10} values correspond to slopes of lines between means at different temperatures (see Prosser, 1973).

semilogarithmically (Fig. 2), a line can be fitted by eye between 5° and 40°C with a slope corresponding to a Q_{10} value of 1.9. The Q_{10} values for temperature intervals within this temperature range vary between 1.3 (30° to 35°C) and 2.9 (5° to 10°C) as is illustrated in Table 1. Ventilation at 0°C was generally very irregular and consisted of a few breaths followed by periods of apnoea lasting for periods up to about ten minutes. As a result of this irregularity, no attempt was made to obtain a mean value at 0°C. There was a significant increase in the ventilatory rate from 35° to 40°C, as is typical of scincids (Dawson, 1960; Hudson and Bertram, 1964). The subjects were, however, neither panting, as described for some iguanids (Dawson and Templeton, 1963), nor gaping without panting, like anguids (Dawson and Templeton, 1966) and agamids (Bartholomew and Tucker, 1963).

Electropneumographic responses to temperature

Use of the orthogonal YY lead made possible the

Fig. 2: Relation of ventilatory rate to body temperature for resting skinks.

Data represent acutely-measured values obtained after at least 30 minutes of equilibration except at 40°C. Range, mean, two standard errors around the mean and number of individuals are represented as in Fig. 1. Range at 0°C extends down to 0.1 breaths/minute (see arrow) and no mean is represented at this temperature (see text for details). A line has been fitted by eye; its slope corresponds to a Q_{10} value of 1.9.

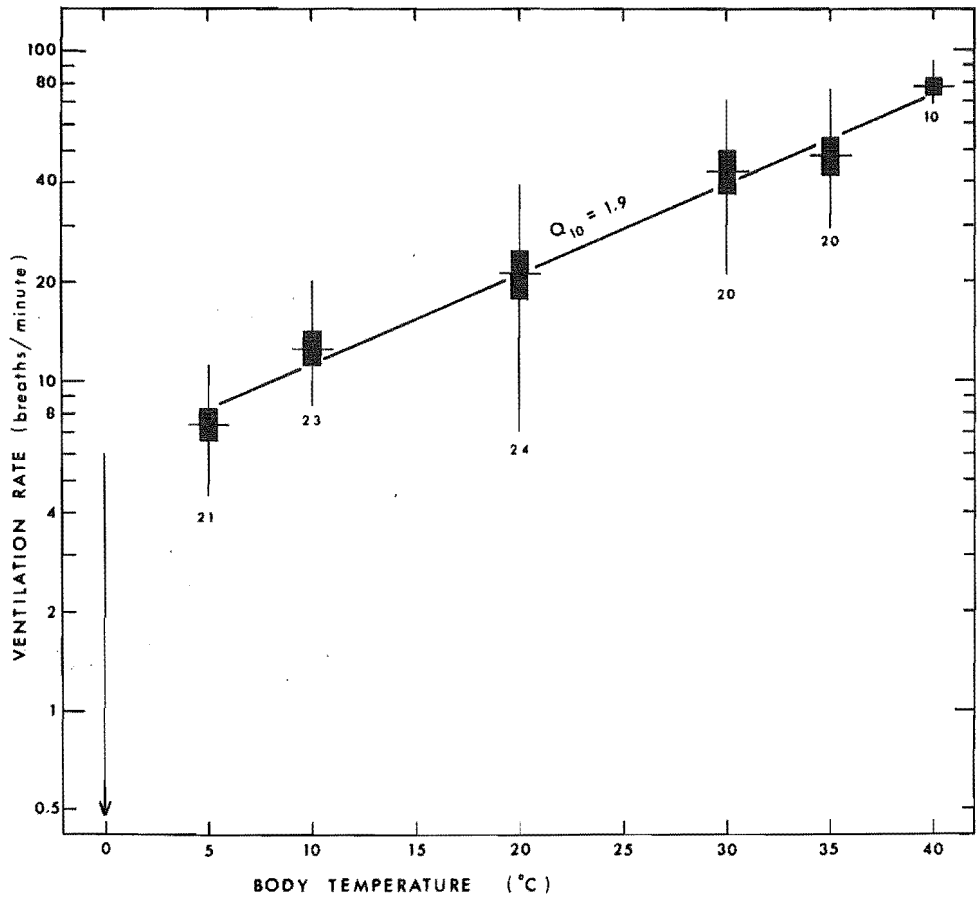

100
80
40
20
10
8
4
2
1
0.5
VENTILATION RATE (breaths/minute)
Q_{10} = 1.9
21
23
24
20
20
10
0
5
10
15
20
25
30
35
40
BODY TEMPERATURE (°C)

Fig. 3: Electroorganograms.

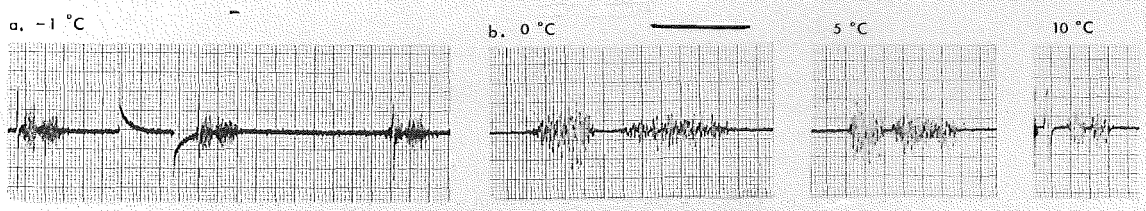
A. Electropneumograms.

a. Record illustrating double bursts of electrical activity associated with active expiration and active inspiration, separated by an electrically quiescent period. Subject was slightly supercooled. Ventilatory and cardiac rates are nearly equal, 3.4/minute. Low frequency cut-off was at 80 hertz. Note electrical differentiation of square wave inserted between two EPG events.
b. Records at three different temperatures from one subject.
Horizontal bars are one second; all records in (b) are at the same paper speed.

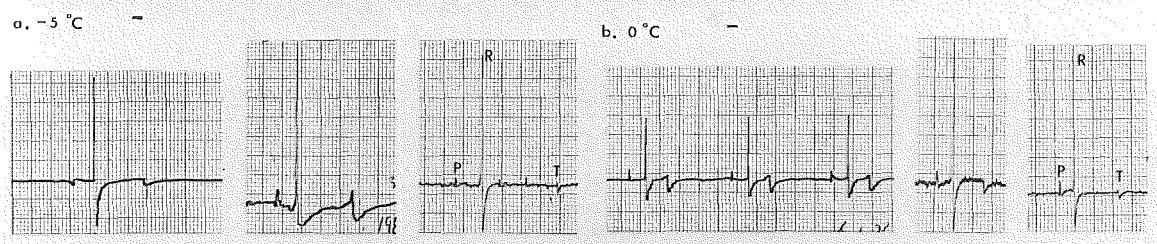
B. Electrocardiograms.

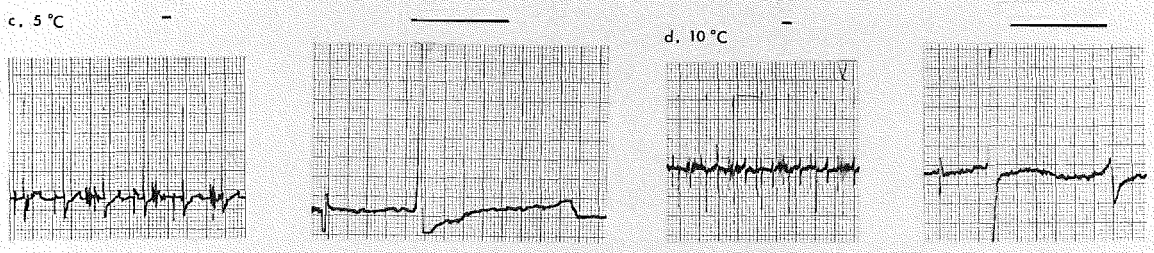
For comparison, records shown are from different subjects and at different temperatures. Major waveforms are labelled in two records.
Horizontal bars are one second and refer to adjacent records (see a, b and e).
ECG in (c) and (d) comprise a low paper speed record followed by a more amplified, high speed record.

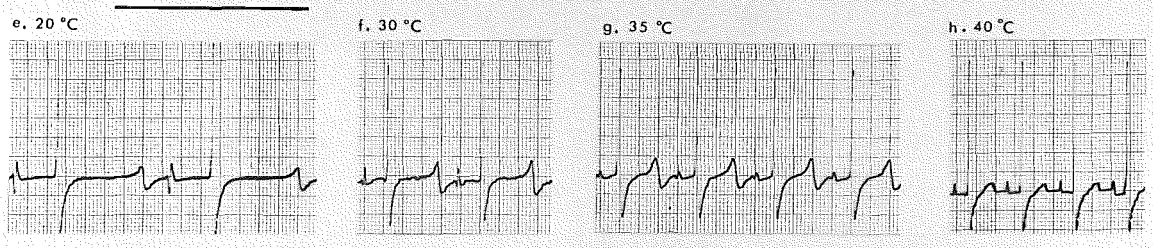
A. ELECTROPNEUMOGRAMS



B. ELECTROCARDIOGRAMS







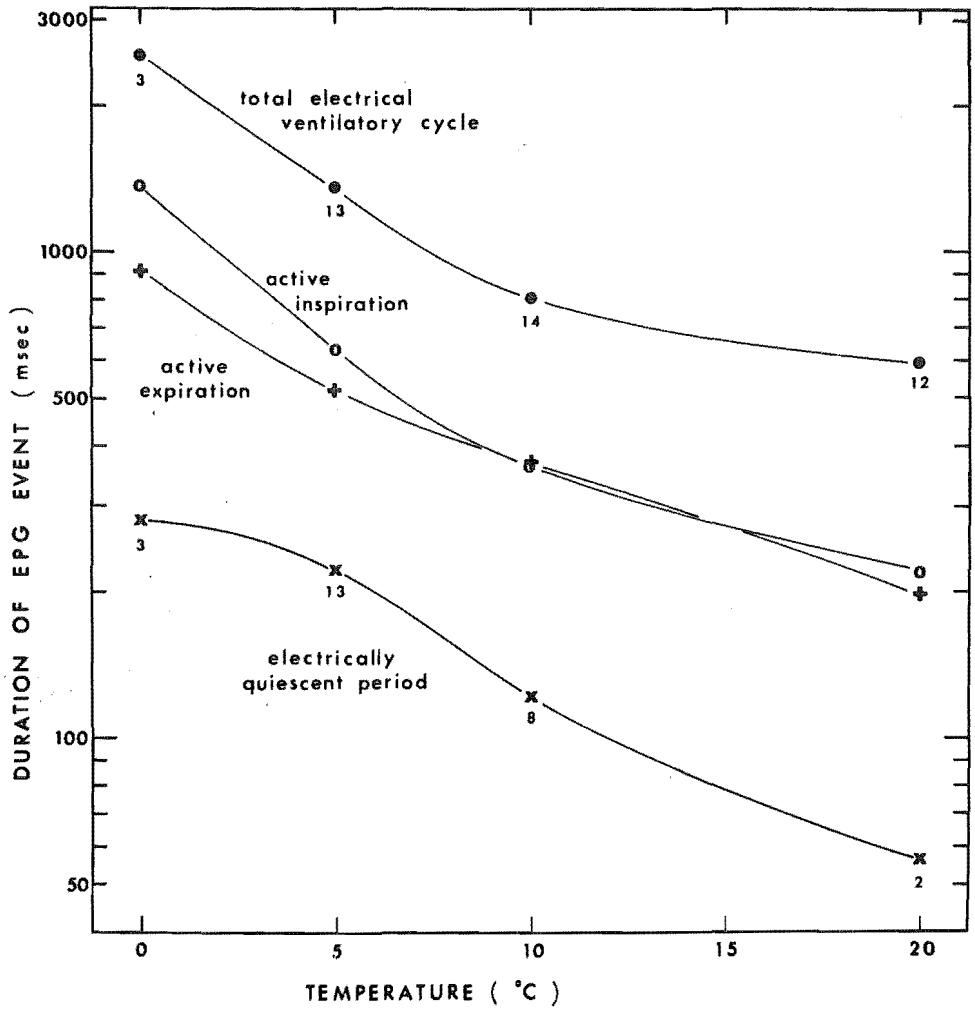
recording of electrical activity of the intercostal muscles associated with ventilation. Such records, referred to as electropneumograms (EPG), were obtained during the same experiments in which ventilatory rates were determined. As illustrated in Fig. 3, EPG typically consisted of double bursts of high frequency electrical activity which correspond to active expiration and active inspiration separated by an electrically quiescent period (see Templeton and Dawson, 1963). To facilitate analysis of EPG, general muscular (EMG) and extraneous noise levels had to be minimal, and the lower frequency signals associated with cardiac activity (ECG) were partially filtered out. At temperatures above 20°C, however, ECG signals increased considerably in amplitude and frequency and generally obscured the EPG signals in spite of filtering. Consequently, EPG from 0° to 20°C only have been analysed. For clarity, only the mean values of the EPG events in this range are plotted semilogarithmically (see Fig. 4). The durations of active expiration and active inspiration are not significantly different ($P > 0.1$) and constitute about equal portions of the duration of the total EPG cycle. The electrically quiescent portion occupies about an eighth of the total EPG duration over the range 0° to 20°C. The Q_{10} values for the reciprocals of the EPG events are presented in Table 2.

Table 2: Q_{10} values for reciprocals of EPG events

Reciprocal of the event	Temperature interval (°C) 0-5	5-10	10-20
Total EPG cycle	3.5	2.7	1.7
Active expiration	3.1	1.9	1.9
Electrically quiescent period	1.6	3.3	2.2
Active inspiration	4.7	3.0	1.7

Fig. 4: Relation of durations of electropneumographic (EPG) events to body temperature for resting skinks.

Data represent mean values; detailed statistics are available (see Morris, 1974; Appendix F). The electrically quiescent period occurs between active expiration and active inspiration (see text for explanation of EPG cycle). Symbols are self-explanatory. Numbers under lowest set of points also refer to the middle two sets of points, and represent numbers of different individuals from which data were obtained. A smooth curve is drawn through means.


3000
1000
500
100
50
DURATION OF EPG EVENT (msec)
total electrical
ventilatory cycle
active
inspiration
active
expiration
electrically
quiescent period
3
13
14
12
3
13
8
2
0
5
10
15
20
TEMPERATURE (°C)

Relation of cardiac rate to temperature

Cardiac rates for resting skinks were acutely measured between -5° and 40°C. When these data are plotted semilogarithmically (Fig. 5), a smooth curve, which has a progressively decreasing slope as temperature increases, can be drawn through the means. The relationship is, however, essentially linear between 20° and 35°C. These trends are reflected in the Q_{10} values for the different temperature intervals (Table 1).

Although the cardiac and oxygen consumption rates could not be measured simultaneously in the Warburg apparatus, the experimental conditions were standardised as much as possible to permit comparison of these responses. In addition, ventilatory rates counted visually during oxygen consumption experiments were comparable to ventilatory rates obtained simultaneously with cardiac rates. The differences in the Q_{10} values for oxygen consumption and cardiac rates (see Table 1) are reflected in the "oxygen pulse", expressed as oxygen consumption per heart beat (Table 3). Oxygen pulse is equal to the product of the stroke volume of the heart and the utilisation coefficient for oxygen (see Dawson, 1967). The increase in the oxygen pulse for this skink at temperatures between 5° and 35°C may indicate an increase in the utilisation of oxygen by the tissues, rather than an increase in the stroke volume of the heart (see Tucker, 1966; Morris, 1974, Paper B). The increase at 0°C, on the other hand, may indicate that the relatively small metabolic requirements of the tissues are met in spite of the very low heart rates. At -5°C, however, the viscosity of the blood would be so high that, although electrocardiac activity persists, blood circulation would probably

Fig. 5: Relation of cardiac rate to body temperature for resting skinks.

Data represent minimum, acutely-measured values which were generally obtained simultaneously with ventilatory rates. Range, mean, two standard errors around the mean and number of different individuals are represented as in Fig. 1. A smooth curve is drawn through means.

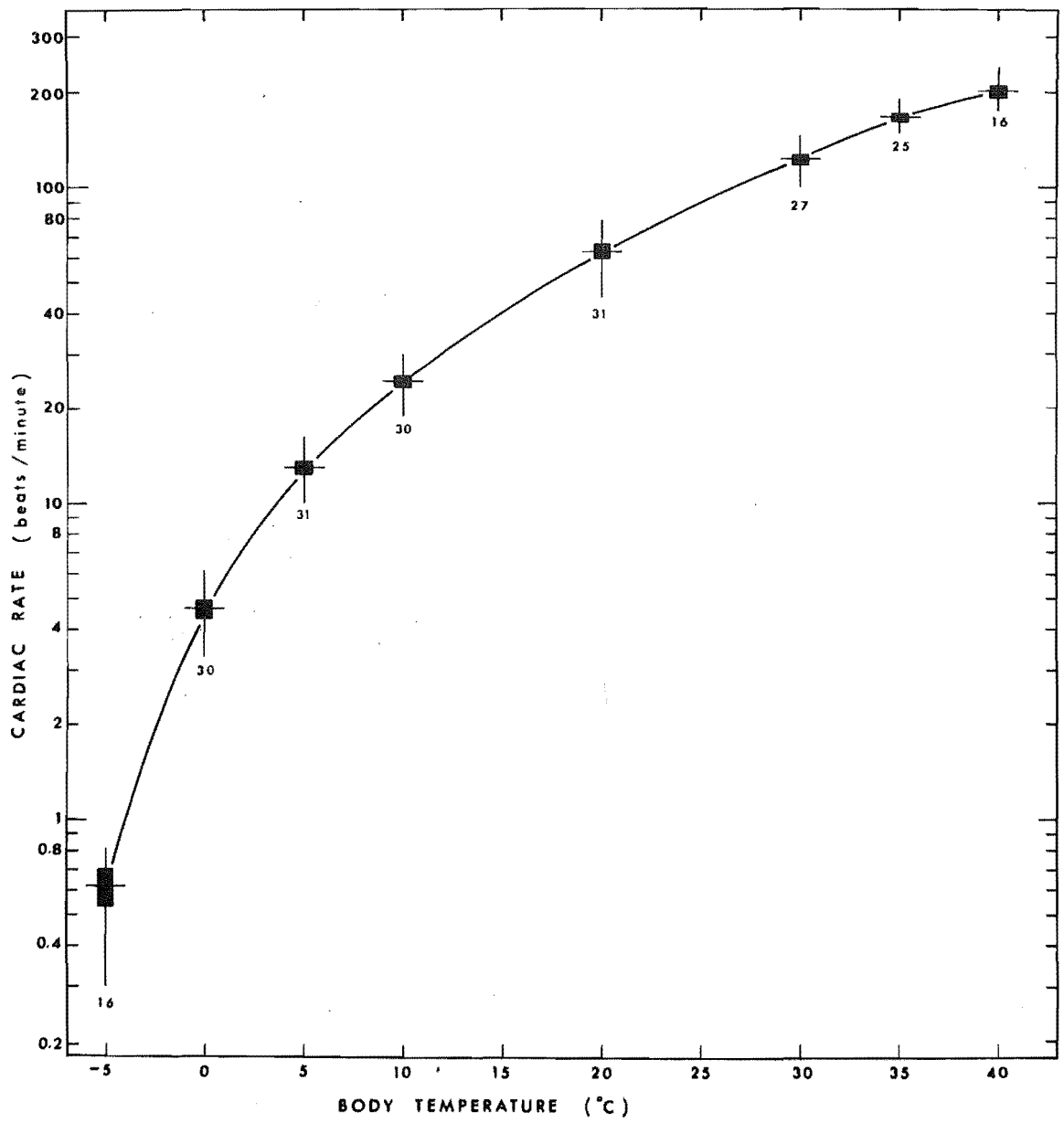

300
200
100
80
40
20
10
8
4
2
1
0.8
0.4
0.2
CARDIAC RATE (beats/minute)
16
30
31
30
31
27
25
16
−5
0
5
10
15
20
25
30
35
40
BODY TEMPERATURE (°C)

Table 3: Oxygen pulse (oxygen consumption per heart beat) for resting skinks at different temperatures

Temperature °C	Oxygen consumption (ml/g/hr)	Cardiac rate (beats/hr)	Oxygen pulse (ml/beat g) x 10^{-5}
-5	0.059	37	16.0
0	0.0130	276	4.7
5	0.0258	775	3.3
10	0.0504	1442	3.5
20	0.1529	3750	4.1
30	0.3299	7320	4.5
35	0.5087	9890	5.1

have ceased. Since no ventilatory activity occurs at this temperature, the oxygen consumed must be diffusing across the body surface.

Two individuals tested at 40°C developed sinu-auricular (SA) block after several minutes at this temperature. The cardiac rates dropped suddenly to 28% and 39% of their initial values. SA block ceased after the subjects were taken from the temperature cabinet. The ECG of each of these skinks was normal when the skinks were cooled and both animals survived the experiment.

Electrocardiographic responses to temperature

Electrocardiograms (ECG) of resting skinks were obtained over the range -5° to 40°C using the orthogonal YY lead described above. The use of platinum electrodes and capacity-coupled amplification produced electrically differentiated waveforms (see Geddes and Baker, 1968). As a result, the R wave and, to a lesser extent, the P and T waves, are biphasic (Fig. 3). Employing the same lead, but with DC

amplification, Valentinuzzi et al. (1969) obtained ECG with monophasic R and T waves and a slightly biphasic P wave for snakes. Differentiation of the waveforms, however, produced sharper deflections, and this facilitated the analysis of the time relations of ECG events. The accuracy of measurement of these events was limited mostly by the clarity of the waveforms. This factor depended on the general noise level and the amplitudes of the waveforms. To facilitate certain measurements, for example, the amplification was increased; this resulted in larger P and T waves and a dampened RS wave (see Fig. 3, ECG (c)).

In this study, the description and interpretation of the ECG events are based on the following criteria. From the onset of the P wave (deflection produced by auricular depolarisation) to the beginning of the RS wave (ventricular depolarisation) is the P-R interval (int.). This interval includes the P wave and the P-R segment (seg.), and is an approximation of the duration of auricular electrical activity. Open chest experiments on L. zelandica have indicated that a small portion of the T wave of the auricles (T_a) would occur during the RS wave (Morris, 1974, Appendix C), hence the P-R seg. gives only an approximation of auricular repolarisation time (except at 40°C, where the T_a wave is generally present in the P-R seg.). The P-R seg. is also a measure of the auriculo-ventricular (AV) conduction time. From the beginning of the RS wave to the end of the T wave is the R-T int. which includes ventricular depolarisation and repolarisation. Hecht (1957) has shown that the R-T int. corresponds to depolarisation and to three phases of recovery (repolarisation) of the ventricular transmembrane potential (TMP). From the beginning of the R wave to the end

of the S wave corresponds to ventricular depolarisation and phase 1 of repolarisation. Phase 2 (the "plateau") and phase 3 of the TMP coincide with the isoelectric portion of the S-T int. and the T wave respectively. The difficulty of establishing the boundaries of these phases, however, has precluded systematic analysis of the S-T int. although certain trends were noted. The P-T int. includes the P-R and R-T int. and represents the duration of electrocardiac activity, although, strictly speaking, it does not include the sinu-auricular conduction time (which cannot be measured on ECG from the body surface of this skink). The R-R int. is the mean duration between successive R waves and, in regular sinus rhythm, it is equal to the P-P int. which includes the electrically-active (P-T int.) and the electrically-inactive (T-P int.) periods of electrocardiac activity. The T-P int. is the mean duration of the period between the end of the T wave and the onset of the P wave. When there is a slight overlap of the P wave on the T wave for example, at 30° and 35°C, there is no T-P int. The duration of the overlap was tabulated as a negative number, although, for statistical purposes, such numbers were treated as zero. The relative durations of various ECG events were determined, including the following: P wave/P-R int., P-R seg./P-T int., RS wave/R-T int., S-T int./P-T int., P-T int./R-R int. and T-P int./R-R int. For clarity, these ratios will be referred to as "mean relative" events, in contrast to the "mean absolute" events, expressed in milliseconds (see McDonald and Heath, 1971).

The effect of temperature on the duration of ECG events is summarised in Fig. 6. The durations of these events vary inversely with temperature over the entire range -5° to 40°C,

Fig. 6: Duration of ECG events in relation to body temperature.

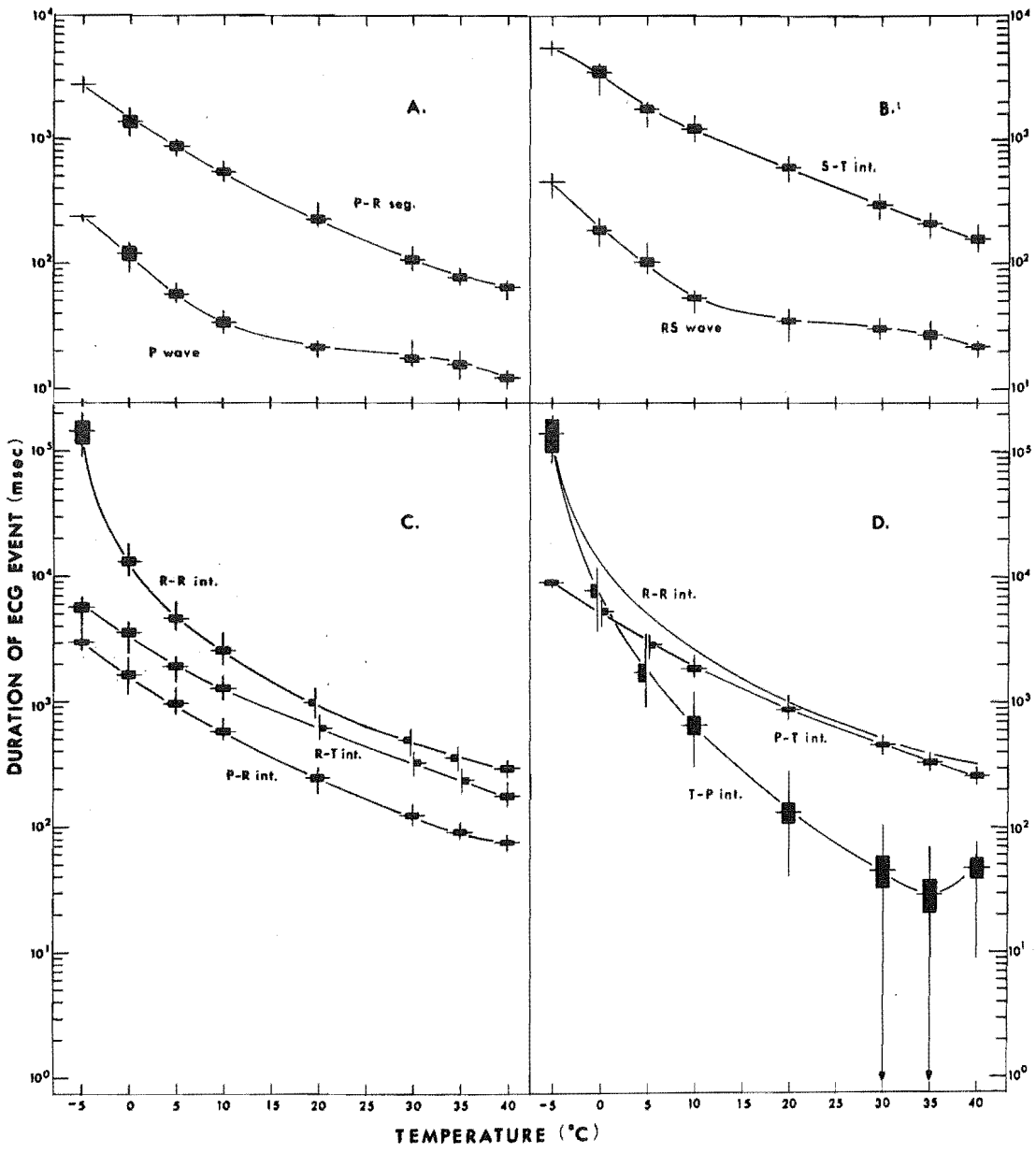
A. Auricular depolarisation (P wave); the delay in conduction across the auriculo-ventricular junction and an approximation of auricular repolarisation (P-R seg.).

B. Ventricular depolarisation (RS wave) and ventricular repolarisation (S-T int.).

C. Total ventricular (R-T int.) and a measure of auricular activity (P-R int.) and the total cardiac cycle (R-R int.).

D. The electrically-active (P-T int.) and electrically-inactive (T-P int.) periods of the ECG cycle; the total ECG cycle (R-R int.) which is shown as the smooth curve fitted in (C).

Descriptions and interpretations of the events are given in more detail in the text. Symbols are as in Fig. 1, except when no standard error is indicated because of a small sample size (A and B), or when ranges of data overlap (C and D). For graphic clarity, the latter symbols are displaced slightly from the experimental temperature and only half of the symbol is represented. Arrows in D indicate that the range continues below 1 msec. In fact, the range cannot be represented on a log scale since the values are slightly negative (see text for details). Also for clarity, the sample sizes are not indicated, but they are available (see Morris, 1974, Appendix F). Smooth curves were fitted by eye using a flexible ruler.


A.
P-R seg.
P wave
B.
S-T int.
RS wave
C.
R-R int.
R-T int.
P-R int.
D.
R-R int.
P-T int.
T-P int.
DURATION OF ECG EVENT (msec)
TEMPERATURE (°C)

except that the T-P int. deviates significantly ($P < 0.05$) from this trend between 35° and 40°C. When reciprocals of the mean absolute events are plotted semilogarithmically, the effect of temperature on the different events can be compared directly. The slopes of the lines drawn between the temperature stations correspond to the Q_{10} values summarised in Table 4.

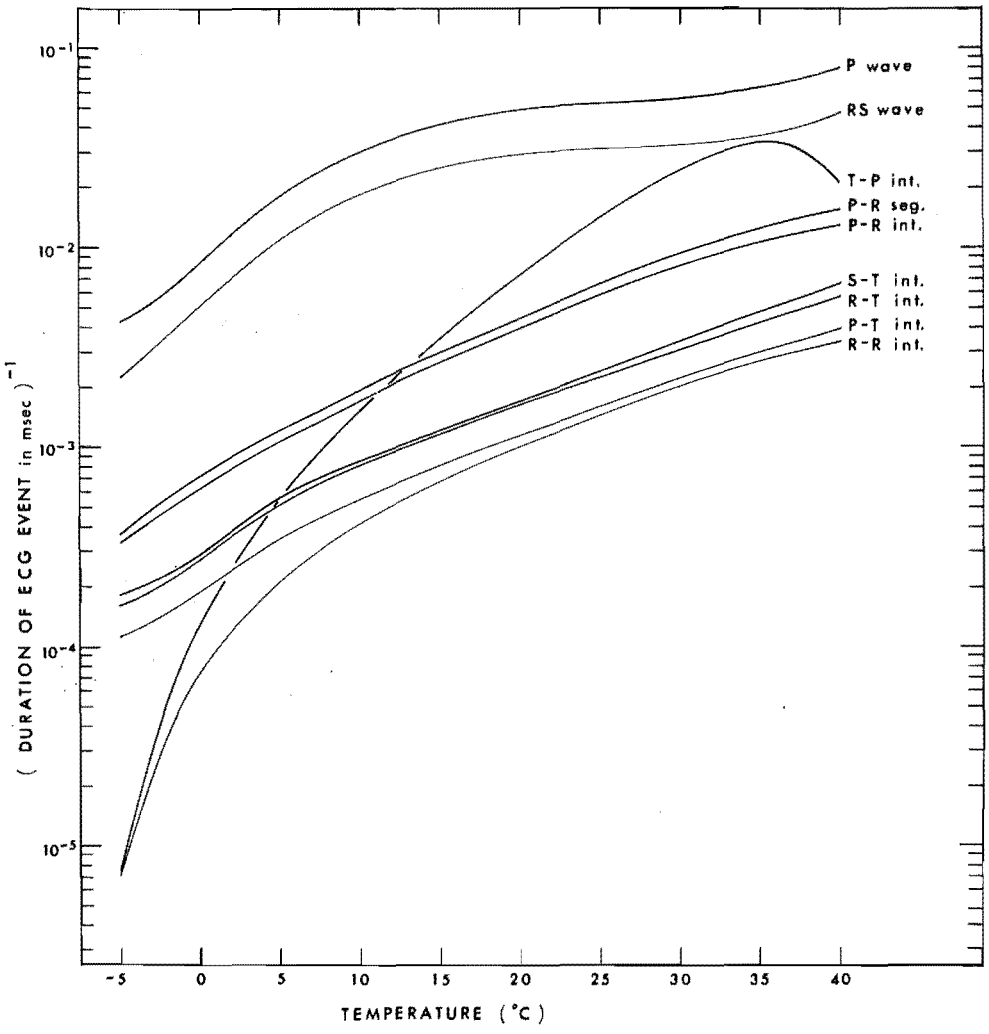
Table 4: Q_{10} values for reciprocals of ECG events of resting skinks.

Reciprocal of the event	Temperature interval (°C)						
	-5 - 0	0-5	5-10	10-20	20-30	30-35	35-40
P wave	3.7	4.4	2.9	1.6	1.2	1.3	1.7
P-R seg.	4.0	2.5	2.6	2.4	2.2	1.9	1.5
P-R int.	3.3	2.8	2.8	2.4	2.0	1.8	1.5
RS wave	5.9	3.3	3.7	1.5	1.2	1.3	1.6
S-T int.	2.4	4.0	2.1	2.1	2.0	2.0	2.0
R-T int.	2.9	3.6	2.3	2.1	1.9	1.9	1.8
P-T int.	2.9	3.4	2.4	2.1	1.9	1.9	1.7
T-P int.	335	18	7.4	5.0	2.9	2.4	0.38
R-R int.	120	8.1	3.2	2.6	2.0	1.9	1.5

The effect of temperature on the duration of depolarisation of cardiac tissue is conspicuously different from its effect on the duration of repolarisation (see Figs. 6 and 7). These differences may be attributed to the fact that depolarisation involves such physical processes as diffusion and conduction, whereas repolarisation involves metabolic (chemical) processes. This point is also illustrated by the Q_{10} values for the respective events at different temperature intervals (Table 4). For example, the Q_{10} values for the reciprocals of the P and RS waves (depolarisation events) are generally greater than 3 below 10°C and less than

Fig. 7: Relation of the reciprocal of ECG events to body temperature for resting skinks.

Events are described in Fig. 5 and in the text. Smooth curves are fitted by eye to the means using a flexible ruler. As a comparison, the slope of the line for the S-T int. from 5° to $40^{\circ}C$ corresponds to a Q_{10} value of about 2.


P wave
RS wave
T-P int.
P-R seg.
P-R int.
S-T int.
R-T int.
P-T int.
R-R int.
(DURATION OF ECG EVENT in msec)$^{-1}$
10^{-1}
10^{-2}
10^{-3}
10^{-4}
10^{-5}
-5
0
5
10
15
20
25
30
35
40
TEMPERATURE (°C)

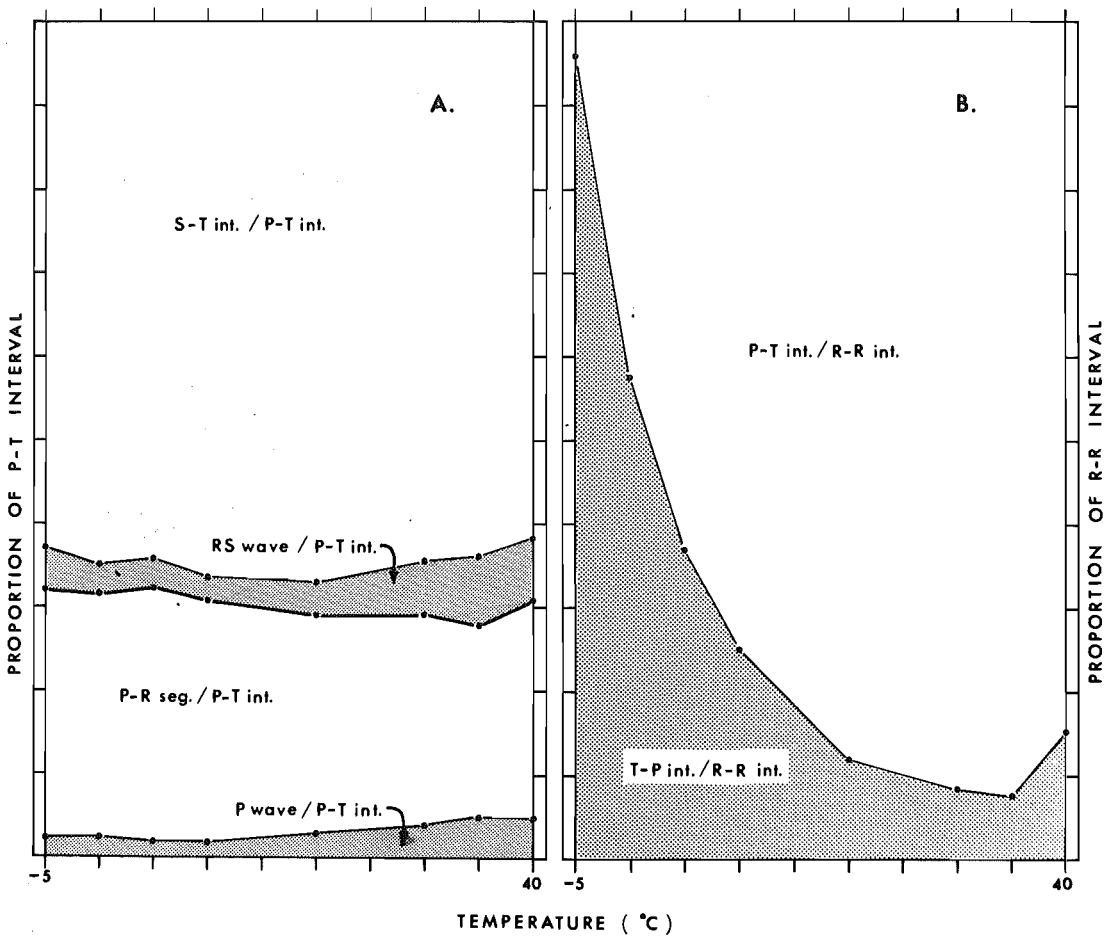
2 above 10°C. Between 20° and 35°C, these events are nearly temperature independent. For the reciprocals of the P-R seg. and S-T int. (repolarisation events), however, the Q_{10} values are between 2 and 3 over most of the temperature range studied. The higher Q_{10} values for most events at lower temperatures indicate a greater thermolability of the corresponding electrocardiac responses at those temperatures. The intrinsic automaticity of the sinus tissue (pacemaker), for example, shows a greater sensitivity to temperatures below 5°C than do the responses of other cardiac tissues. The sensitivity of the pacemaker and/or the SA junction is reflected in high Q_{10} values for the cardiac rate and for the reciprocal of the R-R int. (compare Tables 1 and 4). The AV conduction time (P-R seg.), however, increases markedly below 0°C. This may suggest that first degree AV block has occurred. Between 35° and 40°C, the P-R seg. does not decrease proportionally to the S-T int., and a T_a wave is generally evident at 40°C. These observations may also indicate first degree AV block. From the ECG records illustrated in Fig. 3, it can be seen that the relative duration of the isoelectric portion of the S-T int. decreases markedly with increase in temperature, whereas the T wave correspondingly increases in its relative duration. The proportions (expressed as percentages) of the three phases of ventricular repolarisation changed from about 8:80:12 at 0°C to about 10:55:35 at 40°C. The significant increase ($P < 0.05$) in the T-P int. between 35° and 40°C can be accounted for by the greater decrease in the S-T int. relative to the decrease of the R-R int. over the same temperature interval. This situation is also reflected in the different Q_{10} values of these two intervals (Table 4).

Fig. 8: A. The effect of temperature on the mean relative events of the electrically-active portion of the electrocardiac cycle (P-T int.)

The ordinate is divided into tenths. Shaded areas represent periods of auricular (P wave) and ventricular (RS wave) depolarisation. Unshaded areas indicate period of AV conduction and auricular repolarisation (P-R seg.) and period of ventricular repolarisation (S-T int.). The proportion above the bold line is a measure of the total ventricular electrical activity (R-T int.), and below this line is an approximation of auricular activity (P-R int.).

B. The effect of temperature on the mean relative events of electrical activity (P-T int.) and inactivity (T-P int.) of the electrocardiac cycle.

The T-P int. is shown as shaded.


A.
B.
S-T int. / P-T int.
RS wave / P-T int.
P-R seg. / P-T int.
P wave / P-T int.
P-T int. / R-R int.
T-P int. / R-R int.
PROPORTION OF P-T INTERVAL
PROPORTION OF R-R INTERVAL
-5
40
-5
40
TEMPERATURE (°C)

The effect of temperature on the mean relative P-R int. and the R-T int. is illustrated in Fig. 8A. The mean proportions of auricular and ventricular electrical activity are essentially constant over the range -5° to 40°C, and are 30.5% and 69.5% of the P-T int., respectively. The relative events of auricular and ventricular depolarisation, however, double over the same temperature range. The mean relative P-T int. decreases markedly at temperatures below about 20°C, from 88% to about 7% at -5°C (see Fig. 8B). Above 20°C, however, the mean relative P-T int. increases slightly to 92% at 35°C, then decreases to about 85% at 40°C.

DISCUSSION

Ecological observations indicate that L. zelandica is eurythermic, i.e. active over a wide range of temperatures. It was of interest, therefore, to investigate certain physiological performances over a broad temperature range with a view to characterising some aspects of physiological adaptation and adjustment in eurythermy.

Standard metabolism

The rate of oxygen consumption of resting lizards is a convenient measure of standard metabolic rate (SMR). In spite of the fact that the SMR of a large number of lizards have been reported (see Dawson, 1967; Templeton, 1970), differences in experimental design, in weights of subjects and in many other factors (see Roberts, 1968) limit the extent to which meaningful comparisons can be made between these published data and those for L. zelandica except, perhaps, at low temperatures. There are, for example, large discrepancies

among data obtained by different workers for a single species, U. stansburiana (see Dawson and Bartholomew, 1956; Halpern and Lowe, 1968; Roberts, 1968). The differences in the SMR and Q_{10} values between 5° and 35°C for this lizard are at least as great as the interspecific differences reported for species with different thermal relations (see Dawson and Templeton, 1966). Until experimental procedures are standardised and made more rigorous, it is unlikely that this physiological parameter will be of much value for comparing species with different thermal preferences.

At low temperatures, the ability of subjects to actively modify their metabolism is curtailed (metabolic scope falls off sharply with temperature (see Templeton, 1970)) so SMR values would not be as greatly affected by experimental design. The SMR at temperatures as low as -5°C have been reported for Eremias arguta (Rodionov, 1938) and U. stansburiana (Halpern and Lowe, 1968). These lizards are similar in size to L. zelandica, but are more thermophilic (Brattstrom, 1965). Over the range -5° to 5°C, the mean SMR for U. stansburiana are slightly lower than those for L. zelandica. The Q_{10} values for the ranges, -5° to 0°C and 0° to 5°C, are about 8 and 5 respectively for U. stansburiana, indicating that this species is more sensitive to supercooling than L. zelandica (see Table 1). Rodionov's data (shown graphically in Kalabukhov, 1960) would yield Q_{10} values of about 3 (-6° to 0°C) and 5 (0° to 10°C), although the accuracy of Rodionov's results at lower temperatures appears doubtful.

It is well established that the SMR (expressed on a per weight basis) varies inversely with the body weights of different lizard species. These weights extend from below one

gram to well over 20,000 grams (see Templeton, 1970). The SMR-weight relationship at 30°C was determined for available data by Bartholomew and Tucker (1964). At 30°C, the SMR of L. zelandica is 57% of the value predicted by their equation. The fact that Lygosoma laterale (= Leiolopisma laterale, see Greer, 1970) (Hudson and Bertram, 1964), G. multicarinatus (Dawson and Templeton, 1966), U. stansburiana (Roberts, 1968), L. lineoocellatum (Morris, 1971) and Anolis acutus (McManus and Nellis, 1973) also fell below their predicted values suggests that a new line will be necessary to fit recent data. Considering both the experimental and procedural differences among studies, as well as the inter-specific differences in weights and thermal relations, it is doubtful whether such an equation based on weights is really of much predictive value.

In spite of the difficulties of comparison alluded to above, certain trends are apparent when the thermal dependence of metabolic rates in resting lizards are considered with respect to the thermal relations of the species. It should first be noted, however, that the procedures used to obtain the Q_{10} values have varied among studies. Most of the Q_{10} values have been determined as tangents to curves drawn through scattergrams (see Dawson and Templeton, 1963). In the present study, on the other hand, the Q_{10} values (Tables 1, 2 and 4) correspond to slopes of lines drawn between means obtained at specific temperatures. These means are based on larger sample sizes than were employed in most other studies, and statistical analyses have been conducted. Nevertheless, trends should be valid regardless of the method used and it is apparent that the Q_{10} values of stenothermic heliophiles generally increase at

temperatures below 10° or 15°C (see Dawson, 1967). This increase is correlated with a marked decrease in the alertness and locomotivity of these species. For L. zelandica the Q_{10} values are between 3 and 4 over the range 0° to 20°C. The conspicuous thermophilic response of this eurytherm at low substrate temperatures is understandable in view of the rapid increase in its metabolic activity over this range. From 20° to 35°C, the Q_{10} values of this skink remain essentially constant at about 2. The larger, sympatric skink, L. lineoocellatum had Q_{10} values of about 2.2 over the entire range 5° to 35°C. Thus, although L. lineoocellatum is also eurythermic, it does not exhibit the large temperature dependence in its metabolic rate that was found for L. zelandica below 20°C. This incongruence may be accounted for by weight differences (three- to four-fold) or by the slight differences in experimental design.

There appears to be no correlation between the type of metabolic response to temperature (when plotted semilogarithmically) and the degree of stenothermy or eurythermy, since responses for some species are rectilinear, whereas for other species with very similar thermal relations the responses appear conspicuously curvilinear (see Dawson, 1967). It would seem, however, that the higher resting metabolic rates obtained for eurytherms at the temperature extremes are reflected in both their capacity for activity at lower temperatures, and their relatively lower heat resistance (see Templeton, 1970).

Ventilatory responses

Ventilation in reptiles has been aptly described as

"apnoeic" (McCutcheon, 1964), since complete ventilatory cycles are separated by periods when there is no breathing. As temperature increases, the period of apnoea decreases until, at very high rates (particularly during panting) ventilation becomes similar to the eupnoea exhibited by endothermic vertebrates.

Although the ventilatory activity of many lizard species has been studied, rates are generally difficult to analyse because of their irregularity and their complex response to temperature. Ventilatory rate is temperature dependent in reptiles (Templeton, 1970), but some species have a relatively wide range of temperature over which little or no increase in the rate occurs; examples include G. multicarinatus (Dawson and Templeton, 1966), Sceloporus jarrovi (Burns, 1971) and Crotaphytus collaris (Templeton and Dawson, 1963). Eurythermic scincids tend to have a narrower range over which low thermal dependence of the rate occurs, for example, 19° to 26°C for Eumeces obsoletus (Dawson, 1960), 26° to 33°C for L. laterale (Hudson and Bertram, 1966) and 30° to 35°C for L. zelandica. At temperatures below 5°C, the ventilatory rate falls off sharply in L. zelandica, and a similar situation was observed for L. lineoocellatum (Morris, 1971). In the eurythermic anguid, G. multicarinatus, a sharp fall-off occurs slightly above 5°C (Dawson and Templeton, 1966). Ventilatory rates for the more thermophilic lizards have not been reported at such low temperatures, but ventilation is already very slow and irregular by 15° or 10°C, and this is commensurate with the general increase in lethargy of stenothermic heliophiles (see Boyer, 1967; Dawson and Templeton, 1963).

Electropneumic responses have been reported for one

other lizard, L. lineoocellatum (Morris, 1971), although only the total duration of electrical activity was measured. The response to temperature showed a trend similar to that obtained for L. zelandica, but the durations were slightly longer for L. lineoocellatum at all temperatures except 0°C. The electropneumic cycle illustrated for Crotaphytus collaris at 25°C (Templeton and Dawson, 1963) has a duration similar to that obtained at 0°C for the skinks. Since the iguanids used in that study weighed from 25-40 grams, weight differences probably account for the disparity. Q_{10} values for the EPG events of L. zelandica tended to increase with decreasing temperature, just as occurs for many other physiological processes. Limited data for the leiolopismids suggest that the EPG events have low temperature dependence above 20°C. Without comparable data for similar-sized stenothermic heliophiles, no generalisations can be made about how these findings relate to eurythermy.

Cardiac responses

Resting cardiac rates, like the physiological performances previously discussed, vary with the weights of lizard species. When identical experimental techniques have been used on species of comparable weights, interspecific differences in cardiac rate have been noted. Some of these differences can be correlated with the thermal relations of the respective species (see Dawson, 1967). By comparison to similar-sized eurytherms, stenothermic heliophiles have lower cardiac rates at temperatures below 10° to 15°C. Cardiac rates of the more eurythermic lizards, G. multicarinatus (Dawson and Templeton, 1966), L. lineoocellatum (Morris, 1971) and

L. zelandica do not decrease markedly until about 5°C. Maintenance of an adequate cardiac output at low temperatures, coupled with a relatively high metabolic rate, probably underlies the ability of eurytherms to remain alert and motile.

Between 20° and 40°C, the temperature coefficients for most species studied are relatively constant. The Q_{10} values have generally ranged from 2.0 to 2.5 (Templeton, 1970; Burns, 1971). For L. zelandica, however, the Q_{10} values over this temperature range decrease from 2.0 to 1.5. The inability of the heart of L. zelandica to increase in rate commensurate with the increasing metabolic demands of the tissues may partly account for the heat sensitivity of this species at 40°C. E. obsoletus, although also eurythermic, is able to tolerate 40°C for several hours; furthermore, its Q_{10} value remains constant above 40°C and rates of 300 beats/minute are attained (Dawson, 1960). Although one L. zelandica subject did reach a comparable rate at 41°C, it died during the experiment. Other subjects that were heated above 40°C developed cardiac arrythmia and other ECG abnormalities.

Oxygen pulses for resting lizards at different temperatures have been summarised (see Dawson, 1967; Templeton, 1970). No pattern has emerged relating oxygen pulse at different temperatures to the thermal relations of lizard species. This may be due to the fact that a number of physiological variables are implicated in the concept of oxygen pulse (see Tucker, 1966). To understand the nature of physiological adaptation at the systemic level for lizards with differing thermal relations, it would be necessary to measure some of these variables.

Although absolute and relative ECG events (see Results)

of a large number of lizard species have been reported, the data were not analysed with respect to temperature and, in addition, the animals were anaesthetised (Mullen, 1967). The durations of the P-R and S-T (or, in some studies, R-T) intervals measured peak-to-peak have been determined for E. obsoletus (Dawson, 1960). C. collaris (Dawson and Templeton, 1963), Sauromalus obesus (Boyer, 1967), Iguana iguana (Moberly, 1968) and for the relict New Zealand reptile, Sphenodon punctatum (McDonald and Heath, 1971). These events, the P-R and S-T (or R-T) intervals, provide approximations of, respectively, the auricular electrical activity and AV conduction time, and the duration of ventricular repolarisation.

Information of a more detailed nature on the time relations of ECG over a wide temperature range is available only for the eurythermic skinks L. lineoocellatum (Morris, 1971) and L. zelandica.

An examination of the relevant literature indicates that the durations of electrocardiac events at a given temperature vary directly with the weights of different species. The Q_{10} values for the reciprocals of the P-R and S-T (or R-T) intervals are remarkably similar in the range 20° to $35^{\circ}C$, irrespective of the criteria of measurement of the events, the different weights and thermal relations. Below $20^{\circ}C$, however, the only stenothermic heliophile studied over as wide a range as 10° to $40^{\circ}C$ (C. collaris), has a conspicuously larger Q_{10} value for the reciprocal of the P-R interval than the eurythermic scincids studied (see Dawson, 1967; Morris, 1971; present study).

Among lizards with different thermal relations, the

reciprocal of the S-T (or R-T) interval did not exhibit a similar temperature dependence below 20°C. However, this event was not determined below 10°C for C. collaris; it is possible, therefore, that a study at lower temperatures would reveal a greater temperature dependence for this event.

L. zelandica and L. lineoocellatum have very similar temperature responses for all the electrocardiac events measured in the range 0° to 35°C, with the exception of the R-R and T-P intervals. Both these events reflect responses of the sinus (pacemaker) tissue to the different temperatures; they, like cardiac rate are weight-dependent. The pacemaker tissue of L. lineoocellatum appears to be more sensitive to temperatures as low as 0°C, although pacemaker activity is not as greatly depressed by temperatures between 30° and 35°C. The cardiac responses of L. lineoocellatum were not studied below 0°C nor above 35°C (Morris, 1971).

At temperatures between 35° and 40°C, the Q_{10} values for the reciprocal of the S-T (or R-T) interval for L. zelandica, E. obsoletus and C. collaris vary between 2.0 and 2.2. For the reciprocal of the P-R interval, Q_{10} values are 1.5 for L. zelandica and 2.1 for the other two species. It should be noted that E. obsoletus is distinctly more eurythermic and less thermophilic than C. collaris (Dawson and Templeton, 1963), and that it is a much larger (about ten times) and more thermophilic skink than L. zelandica. These differences preclude a clear distinction between stenothermic heliophiles and eurytherms in terms of high temperature electrocardiac responses.

Whilst some interesting points have emerged from the more detailed studies of time relations of ECG events of the

leiolopismids, the adaptive significance of the variations observed between these and other lizard species cannot be assessed until more data are available.

Acclimatisation

Only a few physiological studies of lizards have involved comparisons of subjects acclimatised (under field conditions) to different seasons (see Mayhew, 1965; Halpern and Lowe, 1968; present study). The limited data available indicate that some stenothermic heliophiles undergo physiological adjustments preparatory to brumation during winter conditions. But for temperate-dwelling lizards which are active throughout the year, physiological changes of a comparable nature do not occur. This statement is based on the finding that resting metabolic rates of L. zelandica and U. stansburiana show no statistically significant seasonal variation. This does not, however, rule out the possibility that some physiological adjustments do occur, since L. zelandica can tolerate temperatures about three degrees higher in the summer than in the winter. Because the maximum voluntary body temperature of this skink is well below the lethal limit, the seasonal difference in heat sensitivity may not be ecologically significant.

A considerable number of studies have involved the acclimation of lizards to various artificial temperature regimes. A few of these experiments have indicated significant differences in metabolic responses between groups acclimated to high and low temperatures, but the physiological mechanisms which underlie these differences are not understood (Dawson, 1967; Templeton, 1970). It is unlikely, however, that under

field conditions temperate-dwelling ectotherms would be exposed to conditions comparable to those used in acclimation experiments.

SUMMARY

1. The main objective of this study was to characterise certain aspects of the thermophysiology of a small (1.3-4.7 g) eurythermic lizard Leiolopisma zelandica. Effects of temperature on oxygen consumption, on ventilatory and cardiac rates, and on the electropneumogram and electrocardiogram of resting skinks were determined. These physiological performances were acutely measured over as much of the range, -5° to 40°C, as was biologically and technically feasible. L. zelandica can tolerate body temperatures as low as -5°C for many hours, but temperatures as high as 40°C for only 15-20 minutes. Voluntary locomotion occurred at body temperatures as low as -1°C.

2. Body temperatures measured in the field ranged from 13.6° to 32.9°C with a mean of 25.2°C (n = 35). When substrate temperatures are low, this skink becomes conspicuously heliothermic, but once warmed it may not adjust its temperature behaviourally except to avoid overheating. L. zelandica is widely distributed (both altitudinally and latitudinally) in New Zealand, and is active throughout the year. On the basis of its thermal relations, this skink has been described as a eurytherm and as an opportunistic or facultative thermophile.

3. Oxygen comsumption of summer- and winter-acclimatised skinks was measured from 0^{o} to $35^{o}C$; there was no significant seasonal difference ($P > 0.1$). In winter, measurements were also made at $-5^{o}C$. Mean rates ranged from 5.9 (at $-5^{o}C$) to 509 $\mu l\ O_2/g/hr$ (at $35^{o}C$). Q_{10} values decrease continuously below $30^{o}C$ from about 2 to 5 (-5^{o} to $0^{o}C$) and increase slightly between 30^{o} and $35^{o}C$.

4. Ventilatory rate was measured between 0^{o} and $40^{o}C$, although at $0^{o}C$ ventilation is generally too sporadic for systematic measurement. Over the range 5^{o} to $40^{o}C$, the mean rates increased from 7.4 to 78 breaths/minute. The overall Q_{10} value between 5^{o} and $40^{o}C$ is about 2, although over intervals within this temperature range the Q_{10} values varied from 2.9 (5^{o} to $10^{o}C$) to 1.3 (30^{o} to $35^{o}C$). Above the range 30^{o} to $35^{o}C$, the Q_{10} value doubles, but panting does not occur. Ventilatory activity was detected as low as $-1.3^{o}C$.

5. Electropneumographic response (electrical activity of the intercostal muscles) was measured between 0^{o} and $20^{o}C$ using an orthogonal YY lead. An electropneumic cycle in this skink consists of a double burst of electrical activity associated with active expiration and active inspiration, separated by an electrically quiescent period. The active events occupy about equal portions of the cycle and the quiescent period constitutes about one-eighth of the cycle. Q_{10} values for the reciprocals of the electropneumographic events have been tabulated.

6. Cardiac rate was measured between -5° and 40°C. Mean rates increased from 0.6 to 201 beats/minute over the range studied. Q_{10} values decrease continuously from 56 (-5° to 0°C) to 1.5 (35° to 40°C). At 40°C, two individuals developed sinu-auricular block and their rates dropped to about one-third of their initial values.

7. Electrocardiograms (ECG) were recorded over the range -5° to 40°C using the orthogonal YY lead. The time relations of conventional ECG events were analysed and found to vary inversely with temperature over the entire range, except that the T-P interval deviated from this pattern between 35° and 40°C. The P and RS waves have similar and complex relations to temperature. Above 10°C, the Q_{10} values for the reciprocals of these waves ranged between 1.2 and 1.7, whereas below 10°C, values as great as 4-6 were obtained. The reciprocals of the P-R segment and the P-R, S-T, R-T and P-T intervals have relatively constant exponential relations to temperature. Most of the Q_{10} values for the reciprocals of these events (i.e. segment and intervals) fell between 2 and 3. The P-R and R-T intervals occupy about 30% and 70% respectively of the P-T interval over the temperature range studied. The sinus (pacemaker) tissue is the most temperature sensitive of the cardiac tissues of L. zelandica, followed by the auriculo-ventricular junction.

8. Since cardiac and metabolic rates were measured under standardised conditions, the amount of oxygen consumed per heart beat (oxygen pulse) could be computed. The oxygen

pulse of this skink is lowest at 5^{o} and $10^{o}C$.

9. The thermophysiological performances characterised for this eurythermic lizard are related to those of other lizards with diverse thermal relations. In particular, the physiological responses of eurytherms and stenothermic heliophiles are compared.

LITERATURE CITED

Bartholomew, G.A. and Tucker, V.A. 1963. Control of changes in body temperature, metabolism and circulation by the agamid lizard Amphibolurus barbatus. Physiol. Zool., 36: 199-218.

Bartholomew, G.A. and Tucker, V.A. 1964. Size, body temperature, thermal conductance, oxygen consumption and heart rate in Australian varanid lizards. Physiol. Zool., 37: 341-354.

Bogert, C.M. 1949. Thermoregulation in reptiles, a factor in evolution. Evolution, 3: 195-211.

Boyer, D.R. 1967. Interaction of temperature and hypoxia on respiratory and cardiac responses in the lizard, Sauromalus obesus. Comp. Biochem. Physiol., 20: 437-447.

Brattstrom, B.H. 1965. Body temperatures of reptiles. Am. Midland Nat., 73: 376-422.

Burns, T.A. 1971. Effect of temperature on heart and ventilatory rates of Sceloporus jarrovi. SWest. Nat., 15: 465-471.

Cunningham, J.D. 1966. Thermal relations of the alligator lizard Gerrhonotus multicarinatus webbi. Herpetologica, 22: 1-7.

Dawson, W.R. 1960. Physiological responses to temperature in the lizard Eumeces obsoletus. Physiol. Zool., 33: 87-103.

Dawson, W.R. 1967. Interspecific variation in physiological responses of lizards to temperature. In Lizard ecology: a symposium (Milstead, W.W. ed.), University of Missouri Press, Columbia, Mo.: 230-269.

Dawson, W.R. and Bartholomew, G.A. 1956. Relation of oxygen consumption to body weight, temperature and temperature acclimation in lizards Uta stansburiana and Sceloporus occidentalis. Physiol. Zool., 29: 40-51.

Dawson, W.R. and Templeton, J.R. 1963. Physiological responses to temperature in the lizard Crotaphytus collaris. Physiol. Zool., 36: 219-236.

Dawson, W.R. and Templeton, J.R. 1966. Physiological responses to temperature in the alligator lizard, Gerrhonotus multicarinatus. Ecology, 47: 759-765.

Geddes, L.A. and Baker, L.E. 1968. Principles of applied biomedical instrumentation. J. Wiley and Sons, New York.

Greer, A.E. 1970. A subfamilial classification of scincid lizards. Bull. Mus. Comp. Zool., 139: 151-184.

Halpern, E.A. and Lowe, C.H. 1968. Metabolism of the agamid lizard Uta stansburiana in the supercooled state. Physiol. Zool., 41: 113-124.

Heath, J.E. 1964. Reptilian thermoregulation : evaluation of field studies. Science, 146: 784-785.

Hecht, H.H. 1957. Normal and abnormal transmembrane potentials of the spontaneously beating heart. Ann. N.Y. Acad. Sci., 65: 700-733.

Hudson, J.W. and Bertram, F.W. 1964. Physiological responses to temperature in the ground skink, Lygosoma laterale. Physiol. Zool., 39: 21-29.

Jensen, D.W. 1972. The effect of temperature on transmission at the neuromuscular junction of the sartorius muscle of Rana pipiens. Comp. Biochem. Physiol. 41A: 685-695.

Kalabukhov, N.I. 1960. The problem of freezing, undercooling and vitrifying of animal organism. In Recent research in freezing and drying (Parkes, A.S. and Smith, A.U. eds) Blackwell Scientific Publications, Oxford: 101-118.

Licht, P., Dawson, W.R. and Shoemaker, V.H. 1966. Heat resistance of some Australian lizards. Copeia, 1966: 162-169.

Licht, P., Dawson, W.R., Shoemaker, V.H. and Main, A.R. 1966. Observations on the thermal relations of Western Australian lizards. Copeia, 1966: 97-110.

Lowe, C.H., Lardner, P.J. and Halpern, E.A. 1971. Supercooling in reptiles and other vertebrates. Comp. Biochem. Physiol., 39A: 125-135.

McCutcheon, F.H. 1964. Organ systems in adaptation: the respiratory system. In Handbook of Physiology, Sect. 4 (Dill, D.B. ed.) Williams and Wilkins, Baltimore, Maryland: 167-191.

McDonald, H.J. and Heath, J.E. 1971. Electrocardiographic observations on the tuatara, Sphenodon punctatus. Comp. Biochem. Physiol., 40A: 881-892.

McManus, J.J. and Nellis, D.W. 1973. Temperature and metabolism of a tropical lizard, Anolis acutus. Comp. Biochem. Physiol. 45A: 403-410.

Mayhew, W.W. 1965. Hibernation in the horned lizard Phrynosoma m'calli. Comp. Biochem. Physiol., 16: 103-120.

Miller, L.K. and Dehlinger, P.J. 1969. Neuromuscular function at low temperatures in frogs from cold and warm climates. Comp. Biochem. Physiol. 28: 915-921.

Moberly, W.R. 1968. The metabolic responses of the common iguana Iguana iguana to activity under restraint. Comp. Biochem. Physiol., 27: 1-20.

Morris, R.W. 1971. On some aspects of the biology, thermal relations and thermophysiology of Leiolopisma lineoocellatum. Unpublished M.Sc. Thesis, University of Canterbury, New Zealand.

Mullen, R.R. 1967. Comparative electrocardiography of the squamata. Physiol. Zool., 40: 114-126.

Prosser, C.L. 1973. Comparative animal physiology. Third edition. W.B. Saunders, Philadelphia.

Roberts, L.A. 1968. Oxygen consumption in the lizard Uta stansburiana. Ecology, 49: 809-819.

Rodionov, W. 1938. On the gas metabolism in reptiles in the state of undercooling (in Russian, with English summary). Soc. nat. Moscow, Sér. biol. Bull. 47: 182-187.

Ruibal, R. and Philibosian, R. 1970. Eurythermy and niche expansion in lizards. Copeia, 1970: 645-653.

Smith, A.U. 1961. Biological effects of freezing and supercooling. Physiol. Soc. Monograph 9. Edward Arnold, London.

Spellerberg, I.F. 1972a. Thermal ecology of allopatric lizards (Sphenomorphus) in southeast Australia. I. The environment and lizard critical temperatures. Oecologia (Berl.) 9: 371-383.

Spellerberg, I.F. 1972b. Thermal ecology of allopatric lizards (Sphenomorphus) in southeast Australia. II. Physiological aspects of thermoregulation. Oecologia (Berl.) 9: 385-398.

Templeton, J.R. 1970. Reptiles. In Comparative physiology of thermoregulation. Vol. I. Invertebrates and non-mammalian vertebrates (Whittow, G.C. ed.) Academic Press, New York: 167-218.

Templeton, J.R. and Dawson, W.R. 1963. Respiration in the lizard Crotaphytus collaris. Physiol. Zool., 36: 104-121.

Tucker, V.A. 1966. Oxygen transport by the circulatory system of the green iguana (Iguana iguana) at different body temperatures. J. exp. Biol., 44: 77-92.

Umbreit, W.W., Burris, R.H. and Stauffer, J.F. 1972. Manometric and biochemical techniques. Burgess, Minneapolis, Minnesota.

Valentinuzzi, M.E., Hoff, H.E. and Geddes, L.A. 1969. Electrocardiograms of the snake: Effect of the location of the electrodes and cardiac vectors. J. Electrocardiology, 2: 245-252.

Veron, J. and Heatwole, H. 1970. Temperature relations of the water skink, Sphenomorphus quoyi. J. Herpetology, 4: 141-153.

Whittow, G.C. ed. 1970. Comparative physiology of thermoregulation. Vol. I. Invertebrates and nonmammalian vertebrates. Academic Press, New York.

CONTENTS

PAPER B Thermophysiological responses of certain tissues *in vitro* of the eurythermic skink *Leiolopisma zelandica* over a wide temperature range.

LIST OF FIGURES

PAPER B

LIST OF TABLES

PAPER B

PAPER B

THERMOPHYSIOLOGICAL RESPONSES OF CERTAIN TISSUES *IN VITRO* OF THE EURYTHERMIC SKINK *LEIOLOPISMA ZELANDICA* OVER A WIDE TEMPERATURE RANGE

INTRODUCTION

Thermophysiological performances of lizard preparations *in vitro* have received only scant attention (Dawson, 1967, 1971; Licht, 1967). Investigations have included the effects of temperature on the contractility of cardiac and skeletal muscle (Licht, Dawson and Shoemaker, 1969), on testicular activity (Licht and Basu, 1967), on glucose metabolism (Beloff-chain and Rookledge, 1970) and on the activity of several different enzymes (Abrahamson and Maher, 1967; Licht, 1967). No studies on the effect of temperature on the oxygen consumption of isolated lizard tissues have been reported, although the effect of thyroxine on tissue metabolism at 30°C has been examined (Maher, 1964). Much of the work at lower levels of organisation has been concerned with responses to temperature above 15° to 20°C and with heat resistance (see Dawson, 1967; Ushakov, 1967), rather than with responses to a broad spectrum of temperature. The contractility of ventricular muscle, however, has been studied over a range as wide as 1° to 50°C (see Licht *et al.*, 1969).

The object of the present study was to determine the effect of temperature on the oxygen consumption *in vitro* of

different tissues of the eurythermic lizard Leiolopisma zelandica. In addition, the isometric twitch tension development of the puboischiotibialis muscle was measured over a wide temperature range.

MATERIALS AND METHODS

Experimental subjects

L. zelandica, the common or garden skink, is probably the most ubiquitous and numerous of the New Zealand reptiles. This skink is widely distributed altitudinally and latitudinally and is active on fine days throughout the year. Experiments have shown that it is capable of voluntary locomotion at body temperatures as low as -1°C and that it can tolerate acute exposure to temperatures as low as -6°C and as high as 41°C. The highest body temperature recorded in the field, however, was 33°C.

The individuals used in this study were captured by hand at Kaitorete Spit (about 40 km south of Christchurch, South Island) and they ranged in weight from 1.5 to 3.4 grams. The skinks were kept in an outdoor terrarium where they were exposed to the prevailing weather and photoperiod, and were provided with water and cover, but were not fed. They were used within a fortnight of capture from the field. All data were obtained between 0900 and 1800 hours during the period January to March 1974, which is coincident with the austral summer and early autumn.

Tissue respirometry

The skinks were collected from the terrarium in the morning before they had begun to bask and were cooled to 0°C.

Dissections and preparation of the tissues were carried out in a cold room at about 4°C. The chilled skinks were decapitated, and up to eight different tissues were excised and placed into separate beakers, each containing ice-cold oxygenated Ringer's solution. The Ringer's solution was based on that of Licht (1964) and consisted of the following: 155 mM NaCl, 4 mM KCl, 2mM $CaCl_2$, 1 mM $MgSO_4$, 5.6 mM glucose and 2 mM Sørensen's phosphate buffer at a pH of 7.2 (glass electrode). Because of the small size of this skink, tissues from as many as ten individuals were pooled for each experiment.

The cranium was sectioned slightly off-centre, exposing the brain which was removed relatively intact. Viscera were then excised, including the heart ventricle, liver, testes (if present), fat bodies, kidneys and intestine (from the pyloric sphinctre to the cloaca). The hind limbs were skinned and the pelvis and limbs extracted together; the bones were dissected out and discarded. Each tissue was blotted on tissue paper to remove excess Ringer's solution, then weighed on tared wax-paper to the nearest mg. The tissues were prepared for respirometry in the most practicable manner: the brain, liver, testes, kidneys and fat bodies were teased apart; the heart and intestine were sliced; and the skeletal muscle was minced freehand. Tissues thus prepared were placed into oxygenated Ringer's solution in calibrated Warburg flasks, and the volumes of the breis made up to 3 ml. The flasks were transferred in an ice bath to another building where the respirometry was conducted.

Oxygen consumption was determined by the direct method with a Braun Warburg Respirometer (Type V85). The flasks and

manometers were calibrated by the mercury method and the flask constants were determined as outlined in Umbreit, Burris and Stauffer (1972). Paper wicks (Whatman No. 1 filter paper) and 0.2 ml of 20% KOH were placed into the centre wells. The flasks were then set into the Braun saline bath which contained a cooling coil and an electrolytic heater. The bath's temperature was regulated to within 0.1°C. The flasks were equilibrated for 15 minutes, during which time they were gassed with oxygen and shaken. Shaking continued during the experiment except when readings were taken.

The duration of the determinations varied with the rate of change of the fluid heights in the manometers at the various temperatures used. A preliminary experiment indicated that the small amounts of tissue, in conjunction with low rates of oxygen consumption, would preclude determinations at 0°C.

To facilitate statistical treatment of the data, determinations were made at six temperature stations within the range 5° to 40°C. Twelve experiments were conducted: seven within the range 20° to 40°C and five within the range 5° to 30°C (and sometimes either 35° or 40°C). In all cases, the experiments commenced from the lowest temperature. For graphical purposes, data have been presented as semilogarithmic plots against temperature and have included the mean, range and standard error of the mean (which is also a measure of the reliability of the data). More detailed statistics are available (Morris, 1974, Appendix F).

Isometric tension development

Subjects were obtained from the outdoor terrarium as

required. They were pithed and the skin was removed from the hind limbs and pelvic region. The puboischiotibialis muscle (see Romer, 1970) was dissected from the thigh by removing all adjacent tissues except the head of the tibia and a fragment of pelvic bone onto which the muscle is attached. Fine wires were tied around the ends of the muscle preparation and were held in place by the bone fragments. The wires were used both to suspend the preparation and to act as stimulating electrodes. The muscle preparation was mounted vertically on a jig which consisted of a glass aerating tube in a Perspex stopper. The wire at the distal end of the preparation was fixed to the bottom of the aerating tube (a recurved hook) while the wire at the proximal end was attached by a thread to a mechano-electrical transducer tube (RCA 5734). The arrangement of the apparatus ensured that the slight tension on the muscle was not altered during transfer of the jig between a pair of cylindrical flasks (see Morris, 1974, Appendix E). These flasks contained Ringer's solution and were immersed in baths, one of which was held at a reference temperature of 20°C, while the other could be varied between 0° and 45°C. Both baths were regulated to within 0.1°C of their set points.

The procedures used in this study were similar to those described by Dawson and Bartholomew (1958) and Licht (1964). The muscle was stimulated by single, widely-spaced pulses from a Grass Stimulator (Model S4G) through a Stimulus Isolation Unit (Model SIU-4B). The isometric tension which developed from the resulting twitches was recorded using the RCA 5734 in conjunction with a Hewlett-Packard DC Preamplifier (Model 350-2700 C) and Paper Recorder (Model 7712 B). Stimulus

parameters (voltage and duration) were adjusted so that the stimuli were supramaximal; this ensured that the tension development at each temperature was maximised.

The reference tension was recorded after the preparation had equilibrated for 15 minutes at 20°C. Subsequently, the preparation was tested from 0° to 40°C (and for a few preparations to 45°C) at five degree intervals, allowing three minutes of equilibration before stimulation. Between each test temperature, the preparation was returned to the reference flask, and the tension was determined to ascertain changes in performance due to such factors as fatigue or heat damage. Oxygen was continuously bubbled through the Ringer's solution in the reference and test flasks.

Following the protocol of earlier workers (see above), the tension development was calculated as a percentage of the preceding reference tension. Then, to permit comparisons among preparations, the highest percentage for each preparation was taken to be 100% and the other percentages were adjusted accordingly.

The total twitch time and the times for the rising phase (contraction) and the falling phase (relaxation) of the twitch were measured from low-speed paper recordings, using a stereo microscope in conjunction with a calibrated eyepiece micrometer.

RESULTS

Oxygen consumption

The rates of oxygen consumption (expressed as µl O_2/g wet weight/hr) of eight different tissues were determined *in vitro* over the range 5° to 40°C (Fig. 1). For all tissues (except skeletal muscle) and over the entire temperature ranges studied, oxygen uptake increased with increasing temperature. From the semilogarithmic plots, it can be seen that the brain is the only tissue which has a rectilinear relationship over the entire temperature range. The responses of liver, kidney and fat body can be fitted to straight lines over the ranges 5° to 20°C and 20° to 40°C. The other tissues - intestine, testis, heart and skeletal muscle - appear to have more complex responses to temperature. The metabolic responses of the various tissues are reflected in the temperature coefficients which are summarised in Table 1.

Table 1: Temperature coefficients (Q_{10} values)* for oxygen consumption of various tissues of *L. zelandica*

Tissue	Temperature interval (°C) 5-10	10-20	20-30	30-35	35-40
Brain	2.0	2.1	2.3	2.2	2.2
Intestine	2.3	3.0	2.0	1.8	1.2
Kidney	2.5	2.8	1.8	1.7	1.6
Heart	2.8	2.0	1.3	1.4	1.5
Testis	1.9	3.3	2.5	2.3	1.7
Liver	1.7	2.3	1.9	1.9	1.6
Fat body	1.9	2.4	1.6	2.0	1.4
Skeletal muscle	1.2	2.2	1.3	1.2	1.2

* Q_{10} values correspond to slopes of lines between means at different temperatures (see Prosser, 1973).

Fig. 1: Oxygen consumption in vitro of eight different tissues of L. zelandica in relation to temperature.

Tissues from up to ten skinks were pooled for each experiment. Seven experiments were run from 20° to 40°C and five from 5° to 20°C or higher. Ranges are represented by the length of vertical lines, means by horizontal lines and a standard error on each side of the means by shaded rectangles. Smooth curves are fitted by eye using a flexible ruler. Open circles (in A, B, G and H) are values reported by Maher (1964) for the skink, Eumeces obsoletus. Shaded circles (in G) are mean values obtained for the frog, Litoria aurea, from two experiments using identical procedures as for the muscles of L. zelandica (see text for details).

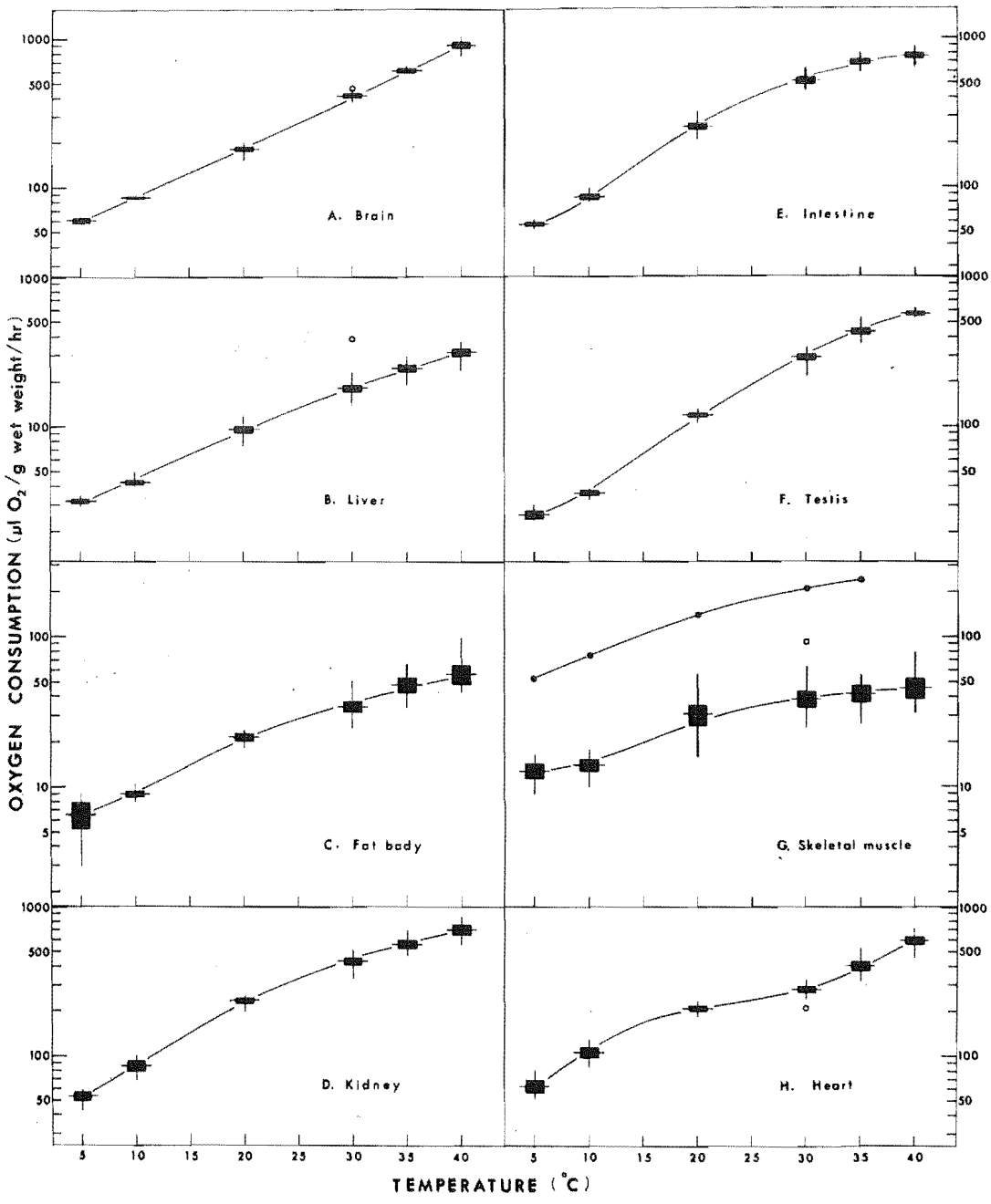
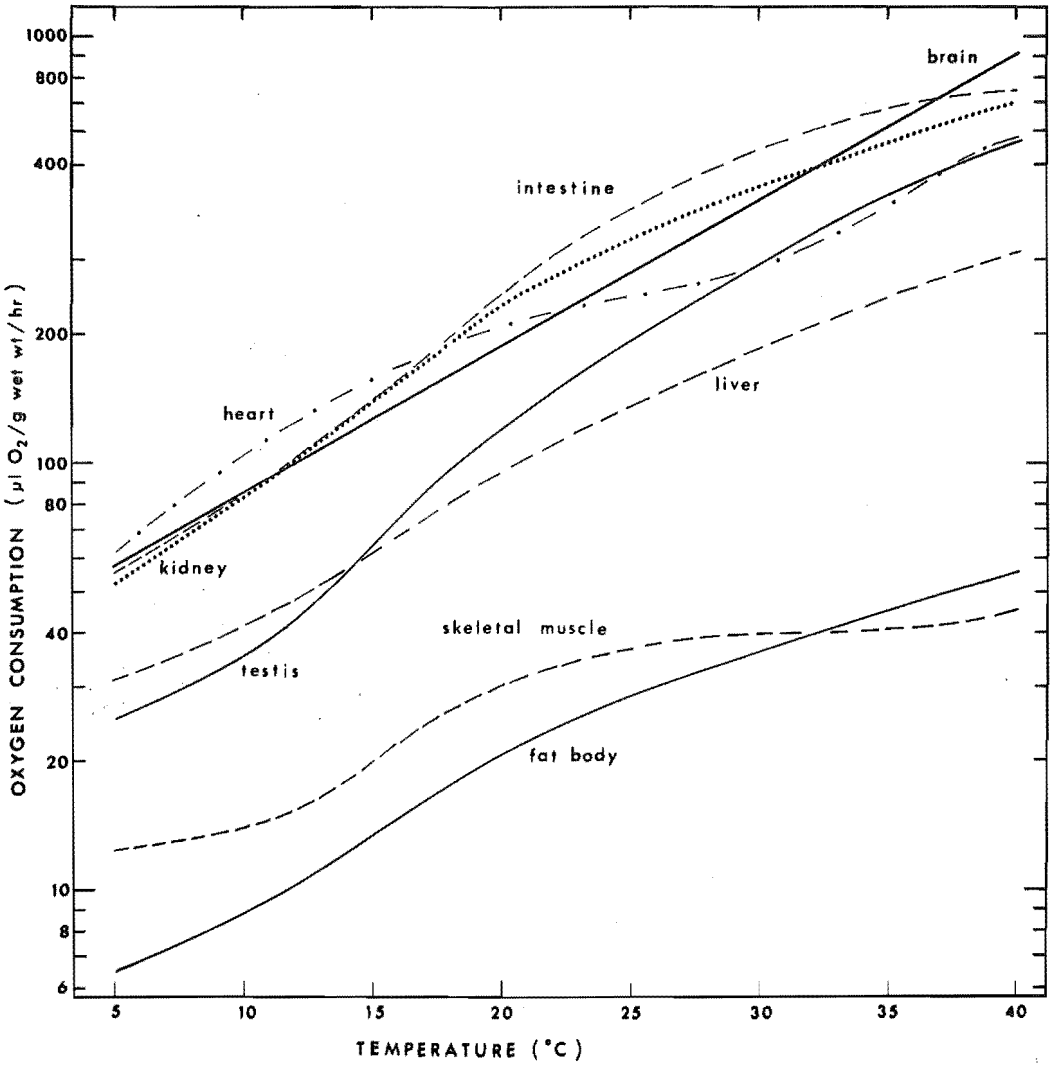

A. Brain
B. Liver
C. Fat body
D. Kidney
E. Intestine
F. Testis
G. Skeletal muscle
H. Heart
OXYGEN CONSUMPTION (µl O2/g wet weight/hr)
TEMPERATURE (°C)

Fig. 2: Comparison of oxygen consumption *in vitro* of eight tissues of *L. zelandica* in relation to temperature

For clarity, different types of lines are used when overlap occurs and the tissues are indicated next to their respective lines. The lines are fitted by eye to data presented in Fig. 1.


1000
800
400
200
100
80
40
20
10
8
6
OXYGEN CONSUMPTION (μl O2/g wet wt/hr)
5
10
15
20
25
30
35
40
TEMPERATURE (°C)
brain
intestine
heart
kidney
liver
skeletal muscle
testis
fat body

When the mean metabolic responses are compared, considerable variation among the eight tissues is evident (Fig. 2). The most active tissues *in vitro* are the brain, heart, kidney and intestine followed by the testis and liver, and the least active are the skeletal muscle and fat body.

Muscle contractility

To obtain a measure of the contractility of excised skeletal muscle, the twitch tension development of the puboischiotibialis muscle was determined. The results of preparations from twelve skinks, obtained over the range 0° to 40°C, are presented in Fig. 3. When the reference tension subsequent to a 40°C measurement was not less than 80% of the preceding reference tension, the preparations (n = 3) were also tested at 45°C.

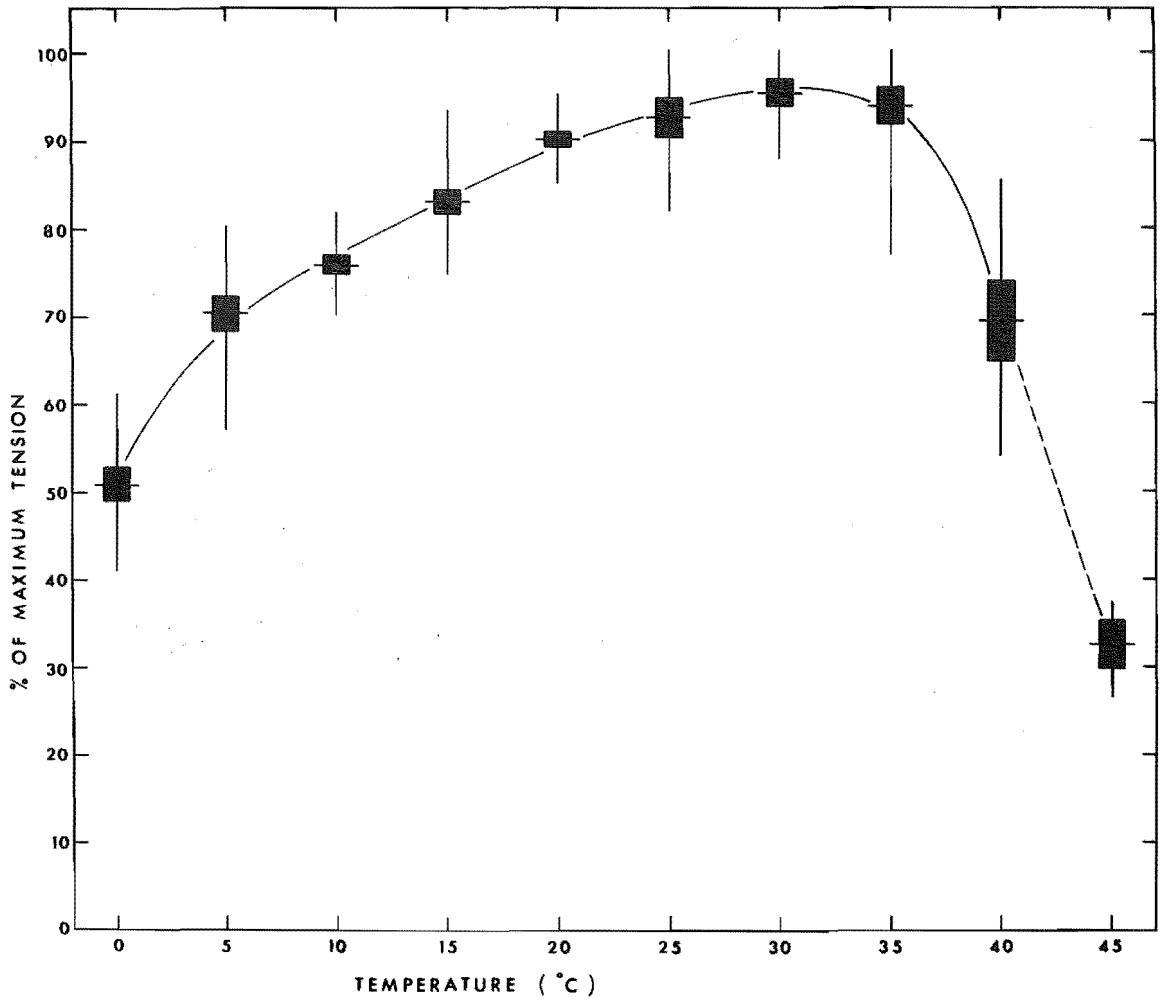
There is no significant difference ($P > 0.1$) in contractility over the range 25° to 35°C, and contractility at 20°C is not significantly different ($P > 0.1$) from 25° and 35°C. Above 35°C and below 5°C, tension development declines rapidly, whereas between 5° and 20°C there is only a gradual increase. Contractility at 5° and 40°C are virtually identical.

Table 2: Mean twitch, contraction and relaxation times of excised puboischiotibialis muscle at different temperatures

Time (seconds)	Temperature (°C) 0	5	10	20
twitch	3.04	1.47	1.00	0.43
contraction	0.95	0.52	0.39	0.19
relaxation	2.09	0.95	0.61	0.24

Fig. 3: Effect of temperature on isometric twitch tension development by excised puboischiotibialis muscle of L. zelandica.

Muscles were stimulated to produce maximal tension after three minutes of equilibration at each test test temperature. Data are represented as percentages of the maximal tension development by individual muscle preparations from 12 skinks between 0^{o} and $40^{o}C$. Three preparations were also measured at $45^{o}C$ (see text). Symbols are as in Fig. 1. Smooth curve is fitted by eye using a flexible ruler.


% OF MAXIMUM TENSION
100
90
80
70
60
50
40
30
20
10
0
0
5
10
15
20
25
30
35
40
45
TEMPERATURE (°C)

Contraction, relaxation and total times of twitches were measured over the range 0° to 20°C. The mean times and the Q_{10} values for these times are presented in Tables 2 and 3. At all temperatures, contraction time is shorter than relaxation time. The larger Q_{10} values for the interval 0° to 5°C indicate a considerably greater temperature dependence than at the higher temperatures.

After testing at 40°C, and particularly after 40°C, twitch times for the reference tension were about two or three times greater than the initial reference tension.

Table 3: Q_{10} values for the reciprocals of twitch, contraction and relaxation times of excised puboischiotibialis muscle

Reciprocal of times	Temperature interval (°C) 0-5	5-10	10-20
twitch	4.3	2.2	2.3
contraction	3.3	1.8	2.1
relaxation	4.8	2.4	2.5

DISCUSSION

Metabolic responses of tissues

There is a paucity of information on the metabolic responses of isolated tissues of vertebrate ectotherms compared to that published for mammals (see Altman and Dittmer, 1968). Tissue metabolism over a broad temperature range has been investigated for relatively few vertebrate tissues and no comparable studies have been reported for reptiles. The present study therefore represents the first measurement of the metabolic activity of isolated saurian tissues over a wide

range of temperatures. Until comparative data become available, attempts to relate experimental results to the eurythermy of L. zelandica must be largely speculative.

Metabolic stability (indicated by Q_{10} values below 2) over a range of temperatures could be advantageous to an ectotherm, since it would impart a measure of physiological independence from environmental temperature fluctuations. Whilst it would seem to be of particular advantage for a eurythermic ectotherm to possess a degree of metabolic stability within its activity range, only the heart and skeletal muscle of L. zelandica appeared to have such a response. The other tissues studied had Q_{10} values which were generally between 2 and 3 over the range 5° to 35°C.

The metabolic response of the whole animal does not correlate well with the tissue responses. The Q_{10} values were 3.8 (5° to 10°C), 3.0 (10° to 20°C), 2.2 (20° to 30°C) and 2.4 (30° to 35°C) (Morris, 1974, Paper A). Presumably the more pronounced response of the whole animal to temperature is related to physiological integration at the levels of the nervous and endocrine systems.

The higher Q_{10} values at lower temperatures which characterise the *in vitro* responses of liver, testis, kidney and heart tissues might have adaptive significance for a eurythermic ectotherm. Relatively low metabolic requirements at low temperatures may allow L. zelandica to conserve its fat reserves and yet should facilitate a rapid increase in metabolic performance of tissues during temperature increases that are achieved by behavioural thermoadjustment.

Although whole animals can tolerate only about 20 minutes at 40°C, no decrease in the metabolic rate was noted during

the course of 40°C trials which lasted up to 45 minutes. It would seem, therefore, that the heat sensitivity of L. zelandica at 40°C must be due to failures of functions at the organ or systemic levels rather than the cellular or subcellular levels.

Metabolic rates at 30°C for the brain, liver, heart and skeletal muscle of the skink Eumeces obsoletus (17-33 g) were determined as controls during a study on the effects of a hormone on metabolic rates (Maher, 1964). The mean metabolic rate for the brain of E. obsoletus is not significantly different ($P > 0.05$) from that for L. zelandica, but the values for liver, heart and skeletal muscle are 2.1, 0.75 and 4.1 times the values obtained for the leiolopismid (see Fig. 1). The major differences in the composition of the medium used by Maher were the 100 mM NaCl and the lack of glucose or other substrate. All the tissues studied were sliced except the brain, which was minced freehand. Tissues were not pooled. Since there is very poor agreement in the literature for identical tissues from mammalian species (see Altman and Dittmer, 1968) it would be rash to attribute the disparity between the results for E. obsoletus and L. zelandica to inter-specific differences.

Skeletal muscle and fat body preparations exhibited the greatest variability in their rates at the various temperatures. The cause of the high variability and the low temperature dependence of the response of skeletal muscle is not clear. To determine whether the shape of the curve and the magnitude of the metabolic rates were due to experimental procedures, comparable tissues from two Australian green frogs (Litoria aurea) were tested. The mean response was slightly curvilinear

over the range 5° to 35°C and the rates were about five times those obtained for L. zelandica (Fig. 1G). For all the vertebrates that have been studied - reptile (Maher, 1964), mammals, amphibians, fish (see Altman and Dittmer, 1968) - skeletal muscle has been one of the least metabolically active of the tissues. Therefore, the response of the skeletal muscle of L. zelandica cannot be dismissed as artefactual.

Fat bodies are largely a storage tissue, hence their low metabolic rate. The variability of some of the data may reflect sampling differences, since the sizes of fat bodies pooled varied considerably. If the relative amounts of inert fat and metabolically-active cytoplasm were not constant between experiments, variability would be expected. Oxygen consumption for white fat of the rat at 37°C is remarkably similar to that obtained for the fat body of L. zelandica at 35° and 40°C. Breibart and Engel (1954) reported a value of 57 ± 6.5 (1 S.E.) for the rat whereas the interpolated value for L. zelandica would be 51 (μl O_2/g wet weight/hr).

The kidney and liver of L. zelandica closely resemble each other in their temperature responses, although the kidney has about twice the rate of the liver. This pattern is not unlike that generally found in mammals, except that the rates for kidney are very much greater than those for liver (see Horwitz, 1964).

Muscle contractility

The general shape of the tension-temperature curve for the skeletal muscle of L. zelandica resembles the curves illustrated for other lizard species (Licht, 1964; Licht et al., 1969). The extent to which comparisons can be made, however,

is limited by the fact that other lizards have not been studied below 16°C.

Muscle tensions of at least 90% of maximum tension occur over the range 20° to 35°C for L. zelandica and tensions of 70% or greater occur between 5° and 40°C. Assuming that in vitro responses of skeletal muscle preparations reflect locomotory responses of intact animals, these experimental findings agree well with the eurythermy of L. zelandica. At 5°C, this skink is still alert and when approached will make relatively effective attempts to evade capture. Skinks at 40°C are capable of very rapid movement, even though in vitro tension development is only about 70% of maximum tension.

Between 5° and 0°C, there is both a sharp drop in tension development and a rapid increase in twitch time. Correspondingly, the capacity of L. zelandica for locomotion falls off markedly over this five degree interval. Although they are still capable of voluntary locomotion at 0°C, their movements are slow and feeble.

The degree of thermophily of the lizards whose muscle contractility has been studied varies considerably (Dawson, 1967). It is of interest to compare the stenothermic heliophiles (Dipsosaurus dorsalis, Uma notata, Physignathus longirostrus, Amphibolurus inermis and A. ornatus) with the more eurythermic lizards (Gerrhonotus multicarinatus, E. obsoletus, Phyllurus milii and L. zelandica) (see Licht, 1964; Licht et al., 1969; present study). The former group are inactivated at body temperatures in the range 15° to 20°C (see Brattstrom, 1965), whereas eurytherms are capable of voluntary locomotion at temperatures well below this. By comparison with the stenothermic heliophiles, the tension-

temperature curves of the eurytherms are consistently displaced towards lower temperatures. The breadth of the temperature range over which at least 90% of the maximum tension develops is, however, similar for eurytherms and stenothermic heliophiles.

The heat sensitivity that is exhibited by eurytherms at the whole animal level is also observed in muscle contractility. For L. zelandica, the extent of heat sensitivity is particularly marked at temperatures greater than 35°C, especially considering that the equilibration periods used were shorter than in studies by other workers.

Although it is possible to draw some correlations between contractility of muscle preparations and locomotory capacities in lizards with different thermal relations, other aspects of the thermophysiology of locomotion need to be investigated. Comparative analyses of the contraction and relaxation times for other lizards might prove fruitful, since the rate at which muscle tension (or muscle length) rises and falls would be an important factor in locomotion. One crucial aspect of locomotivity at different temperatures that has been entirely neglected is the role of the nervous system. In vivo, the nature of nervous input will strictly limit the capacity for muscular output. Therefore, it would probably be very worthwhile to investigate the effect of temperature on contractility, using nerve-muscle preparations. It should be pointed out that although D. dorsalis develops 70% of its maximum tension at 18°C (Licht, 1964), it has been reported to be completely inactivated and brumating (see Mayhew, 1965) at this temperature (Brattstrom, 1965). Conversely, L. zelandica is capable of very effective locomotion at 5°C, a temperature

at which it develops only 70% of its maximum tension. These observations strongly suggest that it is factors other than tension development which limit locomotivity at low temperatures. The possibility that the central and/or peripheral nervous systems are implicated should not be ignored.

SUMMARY

1. The oxygen consumption *in vitro* for eight different tissues of the eurythermic skink *Leiolopisma zelandica* was measured over the range 5° to 40°C. The tissues employed were brain, intestine, liver, kidney, testis, heart (ventricle), fat body and skeletal muscle. Isometric twitch tension development of the puboischiotibialis of this skink was investigated over the range 0° to 40°C for preparations from twelve skinks and at 45°C for three preparations.

2. The metabolic responses increase with increasing temperature for all tissues studied except skeletal muscle. There are significant differences between the tissues in oxygen uptake *in vitro* both in terms of their absolute values (µl O_2/g wet weight/hr) and temperature dependence (Q_{10} values). The Q_{10} values are generally between 2 and 3 within the range 5° to 35°C, except for skeletal and ventricular muscle. The latter tissues have responses which are relatively temperature independent above 20°C. The most active tissues *in vitro* are the brain, intestine, kidney and heart, followed by testis and liver, whilst skeletal muscle and fat body are the least active tissues.

3. Contractility of skeletal muscle is relatively linear over the range 20° to 35°C, but falls off gradually below 20°C and precipitously above 35°C. Contraction and relaxation times (which were measured from 0° to 20°C) are most sensitive to temperature below 5°C.

4. The implications of tissue thermophysiology are discussed, where possible, with relation to eurythermy.

LITERATURE CITED

Abrahamson, Y. and Maher, M. 1967. The effects of temperature upon pancreatic amylase in selected reptiles and an amphibian (Rana pipien, Eumeces obsoletus, Dipsosaurus dorsalis). Can. J. Zool., 45: 227-232.

Altman, P.L. and Dittmer, D.S. eds 1968. Metabolism. Fed. Am. Soc. Exp. Biol., Bethseda, Maryland.

Beloff-chain, A. and Rookledge, K.A. 1970. A comparative study on the influence of temperature on the metabolism of glucose in isolated reptilian and mammalian muscle. Comp. Biochem. Physiol. 37: 67-72.

Brattstrom, B.H. 1965. Body temperatures of reptiles. Am. Midland Nat., 73: 376-422.

Breibart, S. and Engel, F.L. 1954. The influence of cortisone and insulin on the respiratory activity of fat adipose tissue in vitro. Endocrinology, 55: 70-76.

Dawson, W.R. 1967. Interspecific variation in physiological responses of lizards to temperature. In Lizard ecology: a symposium (Milstead, W.W. ed.) University of Missouri Press, Mo: 230-269.

Dawson, W.R. 1971. Reptiles as research models in comparative physiology. J. Am. Vet. Med. Assoc., 159: 1653-1661.

Dawson, W.R. and Bartholomew, G.A. 1958. Metabolic and cardiac responses to temperature in the lizard Dipsosaurus dorsalis. Physiol. Zool., 31: 100-111.

Horwitz, B.A. 1964. Temperature effects on oxygen uptake of liver and kidney tissues of a hibernating and non-hibernating mammal. Physiol. Zool., 37: 231-239.

Licht, P. 1964. A comparative study of the thermal dependence of contractility in saurian skeletal muscle. Comp. Biochem. Physiol., 13: 27-34.

Licht, P. 1967. Thermal adaptation in the enzymes of lizards in relation to preferred body temperature. In Molecular mechanisms of temperature adaptation (Prosser, C.L. ed.) Academic Press, N.Y.: 131-145.

Licht, P. and Basu, S.L. 1967. Influence of temperature on lizard testes. Nature, 213: 672.

Licht, P., Dawson, W.R. and Shoemaker, V.H. 1969. Thermal adjustments in cardiac and skeletal muscles of lizards. Z. vergl. Physiologie, 65: 1-14.

Maher, M.J. 1964. Metabolic response of isolated lizard tissues to thyroxine administered in vivo. Endocrinology, 74: 994-995.

Mayhew, W.W. 1965. Hibernation in the horned lizard Phrynosoma m'calli. Comp. Biochem. Physiol., 16: 103-120.

Prosser, C.L. 1973. Comparative animal physiology. Third edition. W.B. Saunders, Philadelphia.

Romer, A.S. 1970. The vertebrate body. Fourth edition. W.B. Saunders, Philadelphia.

Umbreit, W.W., Burris, R.H. and Stauffer, J.F. 1972. Manometric and biochemical techniques. Fifth edition. Burgess, Minneapolis, Minnesota.

Ushakov, B.P. 1967. Coupled evolutionary changes in protein thermostability. In Molecular mechanisms of temperature adaptation (Prosser, C.L. ed.) Academic Press, N.Y.: 107-131.

SECTION 2

PART A Some aspects of the biology and ecology of *Leiolopisma zelandica*.

PART B Some aspects of the behaviour and physiology of *Leiolopisma zelandica* at temperature extremes.

CONTENTS

PART A Some aspects of the biology and ecology of *Leiolopisma zelandica*

LIST OF FIGURES

PART A

PART A

SOME ASPECTS OF THE BIOLOGY AND ECOLOGY OF LEIOLOPISMA ZELANDICA (GRAY, 1843)

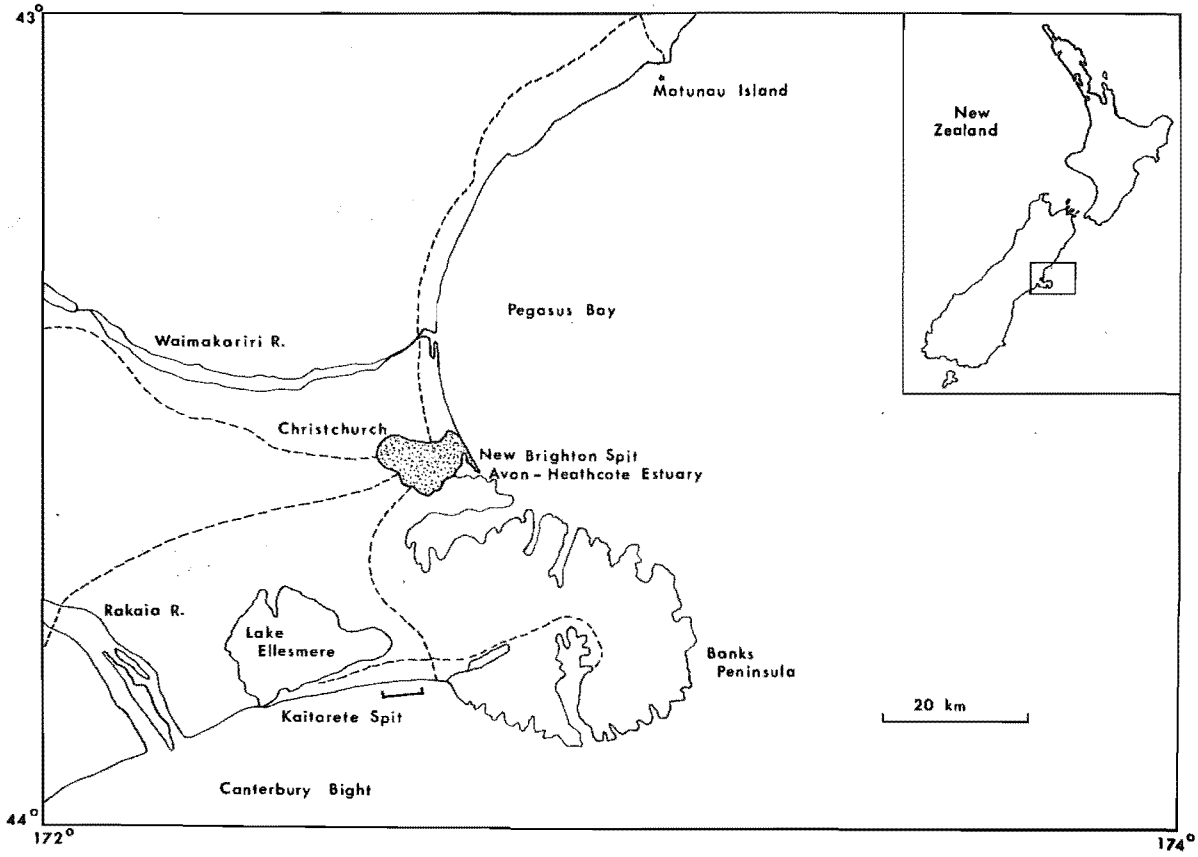
INTRODUCTION

The common or garden skink, Leiolopisma zelandica, is probably the most numerous and ubiquitous of New Zealand's reptiles. On the South Island, it is found on the sea coast, across the plains and on the mountains. Its range extends as far south as 47°S latitude (Stewart Island). McCann (1955) has reviewed the systematic status of the species. The general morphology and life history have also been described in some detail (Barwick, 1959). Most of his information was obtained from a relatively isolated population living in a Wellington cemetery (North Island).

To provide subjects for a study of the thermophysiology of L. zelandica, large numbers of individuals were required. These were collected from Kaitorete Spit and, to a lesser extent, from the Christchurch area (see Fig. 1). Field observations were made during these collecting forays and during a short visit to Motunau Island (November 8-11, 1970). Additional observations were obtained from individuals kept in an outdoor terrarium (see Appendix A) on the roof of the Zoology Building. The conditions in the terrarium simulated those at Kaitorete Spit where most of the lizards were

Fig. 1: Study areas.

The three study areas mentioned in this report are Motunau Island, a marshy area on the New Brighton Spit and Kaitorete Spit (section of dunes between square bracket). The stippled area is Christchurch and the dashed lines represent major roads.


43°
Motunau Island
New Zealand
Pegasus Bay
Waimakariri R.
Christchurch
New Brighton Spit
Avon - Heathcote Estuary
Rakaia R.
Lake Ellesmere
Banks Peninsula
20 km
Kaitorete Spit
Canterbury Bight
44°
172°
174°

collected. Certain aspects of their biology were examined further in the laboratory.

The object of this report is to present these data and observations, and to discuss the thermal relations of this small, successful New Zealand lizard.

STUDY AREAS

Field observations were obtained from three coastal areas which are exposed to similar macroclimatic conditions but which differ considerably in terms of their substrate and vegetation, and in the availability of cover and food for lizards.

Motunau Island has an area of about 3.6 hectares and is situated about a kilometre off the Canterbury coast (see Fig. 1). It consists of a plateau about 30 metres above sea level with sides ranging from sheer cliffs to steep slopes. This small island is an important breeding ground for large populations of several species of sea birds. For this reason and others, it has been created a Reserve for the Preservation of Flora and Fauna. The sea birds live in burrows on the plateau and on the slopes. The other conspicuous group of vertebrates on the island is three species of lizards: L. zelandica, L. lineoocellatum and Hoplodactylus pacificus (Gekkonidae). The predominant vegetation is silver tussock (Poa caespitosa), ice plant (Disphyma australe) and barley grass (Hordeum murinum). For more details about the physiognomy and the flora and fauna of this island see Burton (1967).

Most of the skinks collected in the Christchurch area

were obtained from a small marsh (tidal wetland) on the New Brighton Spit (see Fig. 1). The marsh is surrounded by a stand of exotic trees (Pinus radiata and Cupressus macrocarpa of Jellicoe Park) and by a recently constructed stopbank on the edge of the Avon-Heathcote Estuary. This relatively isolated pocket (less than a hectare) of tidal wetland is covered with salt-marsh ribbonwood (Plagianthus divaricatus) which is more than a metre in height. Sea rush (Juncus maritimus) and fescue (Festuca arundinacea) grow in patches among the ribbonwood. To protect this unusual plant association, the Botany Department of the University of Canterbury has proposed to the Christchurch City Council that this area and other wetland areas around the estuary be designated as Nature Reserves (Dr C. Burrows, pers. comm.). L. zelandica is the only lizard found in this area.

Kaitorete Spit was selected as the major collecting area for subjects for the thermophysiological study of L. zelandica because they were relatively numerous and easy to catch. This spit is bordered by the Pacific Ocean on the south and Lake Ellesmere on the north (see Fig. 1) and is characterised by a steep shingle beach, young dunes and a series of flat terraces of older shingle extending to the shallow lake. The habitat at the spit is interesting because of its relative simplicity in terms of substrate and of floral and faunal components (see Morris, 1971). The same three species of lizard that are found at Motunau Island also occur on this spit. Although these lizards occurred over most of the spit, L. zelandica was captured mainly on the sand dunes. The dunes are sparsely covered with the indigenous, sand-stabilising sedge, Desmoschoenus spiralis (pingao). Near the

beach, there are also accumulations of driftwood and other debris.

STUDY ANIMAL

General field observations

L. zelandica, like many other scincid species, is a diurnal, ground-dwelling lizard which spends most of its time under cover. This skink appears to prefer the more open habitats (rather than forests) where it ranges widely amongst the vegetation. It utilises a wide variety of ground cover and its density may well be limited in some areas by the availability of cover. At Motunau Island, the lizards live in burrows of the sea birds and in the vegetation which occurs on the plateau and on some of the slopes. They are also found under rocks and other debris on a grass-covered storm beach on the south-east side of the island. At the New Brighton Spit habitat, L. zelandica is found in or under rotten logs scattered throughout the area and in the low-growing vegetation. Because this area is often inundated with water, the logs are probably the major cover, while the vegetation is used for foraging. The skink occupies tunnels made in the logs by sand scarab larvae (Pericoptus truncatus) which are larger in diameter than the lizards. The larvae are also found in and under the driftwood at Kaitorete Spit, where they are utilised as food by L. lineoocellatum (Morris, 1971). On the sand dunes at Kaitorete Spit both of the skinks are found in the pingao and under driftwood. They are adept at burrowing into loose substrate (such as the sand at the spit) as a means of evading capture and avoiding thermal stress. Their long, flexible

streamlined bodies with muscular necks and pointed heads, and their movable eyelids with transparent windows (palpebral discs) are important adaptations for burrowing (see Bellairs, 1969). Because of its propensity for burrowing beneath the sand at Kaitorete Spit during periods of inactivity, L. zelandica could only be collected when active. Individuals were generally captured as they darted amongst the pingao after their cover was disturbed. When in broken terrain or thick vegetation, this skink uses its limbs for locomotion. On sand or in water, however, rapid locomotion is accomplished with snake-like movements involving little use of limbs, except as pivots to alter its direction. Effective concealment during inactivity, in combination with covertness, alertness and celerity during activity, may account for some of the success of L. zelandica and its cosmopolitan congeners.

Growth and reproduction

L. zelandica is viviparous, as are all but one species (L. suteri) of New Zealand lizards. At Kaitorete Spit, young are born from about the first week in December through to February, although most appear from late December to early January. These dates are about a month earlier than those reported by Barwick (1959) for his Wellington population.

Young born in the laboratory, even those from the same litter, varied considerably in snout-vent length (SVL) and weight at birth. Although young born early in the season double their birth weight by the end of the first growing season (about March), their SVL increases by only about 50%. Then, until the beginning of the next growing season (about

September), there is little or no further growth. Notwithstanding Barwick's conclusions about the growth of L. zelandica (which were based on similar observations), this species of skink is relatively slow growing. Comparable-sized North American skinks and iguanids such as Eumeces fasciatus (Fitch, 1954), Lygosoma (= Leiolopisma; see Greer, 1970) laterale (Tinkle, 1967) and Uta stansburiana (Fitch, 1967) grow to about twice their birth SVL during the first growing period.

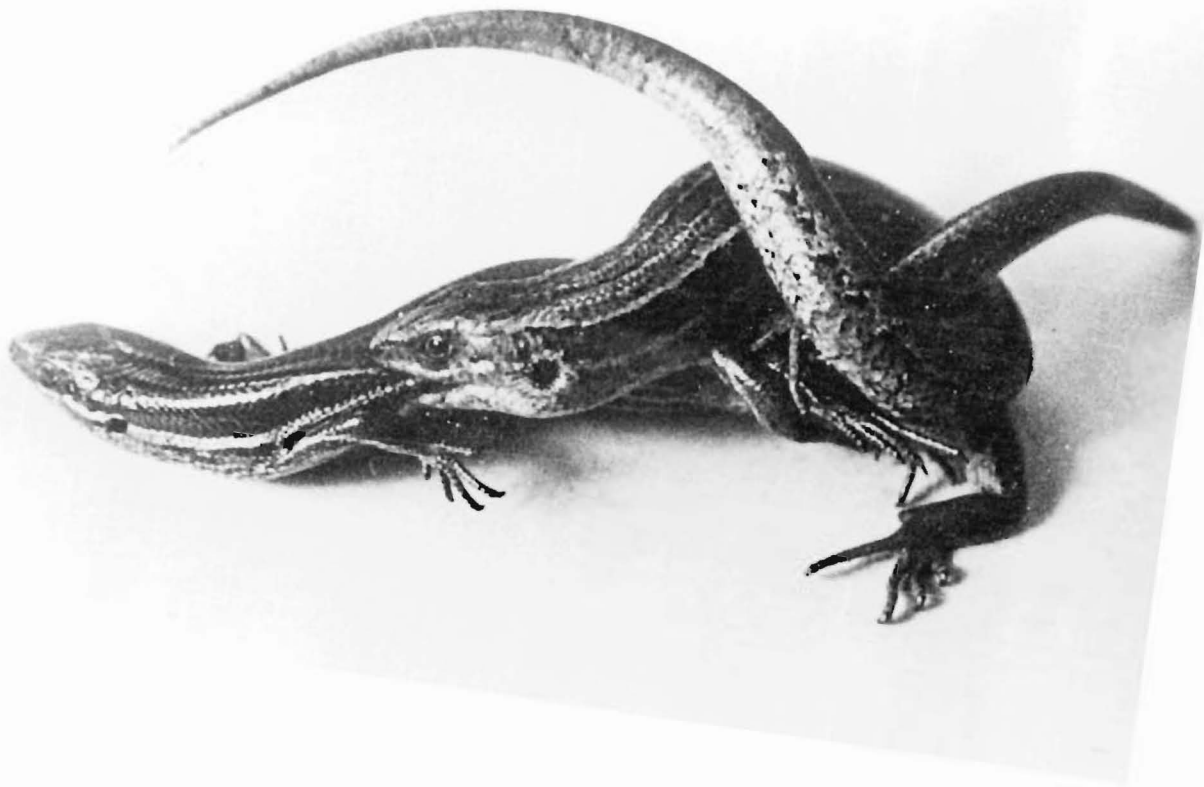
Although active individuals captured at the spit during the winter had very little in their guts, it would seem that a lack of food is not what limits growth. Individuals that were kept in the laboratory (which was exposed to the normal winter photoperiod) and were provided with food ad libitum and basking facilities, also showed little or no weight gain. Moreover, those individuals which had dropped their tails during the autumn or winter formed scabs but did not regenerate their tails until spring. These observations suggest that photoperiod rather than food availability per se regulates growth.

L. zelandica is also slow to mature sexually. Using the presence of sperm or ovarian follicles as indications of sexual maturity, it was found that males and females were not sexually mature until after their third growing season. At that stage they would be more than two years of age. By comparison, L. laterale (Brooks, 1967) and U. stansburiana (Tinkle, 1967) are able to produce young within their first year.

Copulation was observed on one occasion (April 30, 1973). Two individuals had been collected from the outdoor terrarium and placed in a container in the laboratory. Several minutes later they were observed as shown in Fig. 2. Barwick's only

Fig. 2: Copulation.

Observation occurred in the laboratory on April 30, 1973. Individuals were fresh from the outdoor terrarium. Slight sexual dimorphism is evident in their head shapes, the male's head is relatively larger.



observation was on March 28 (1955) which is also coincident with the austral autumn, but he suggested that the copulation was probably displacement behaviour. In spite of the fact that sperm are abundant in the epididymis during the autumn and winter, but absent during the spring (Barwick, 1959; personal observation), Barwick concluded that normal copulation occurs during the spring. This conclusion hardly seems justified. It appears far more likely that copulation occurs in the autumn and early winter period and that the sperm are stored until the eggs move into the oviducts.

Ovarian follicles increase in size during the autumn and winter, and at the spit ovulation occurs during the first few weeks in October. The total number of embryos found in a gravid female ranged from two to five, but three was most common. The fecundity of the population at the spit was lower than that of the Wellington population in which a litter size of four to six was not uncommon (Barwick, 1959). Development in the oviduct must be relatively rapid, taking about 8-10 weeks, since young are already abundant by late December. Barwick, on the other hand, has suggested that the gestation period is about three months.

The advantages of viviparity over oviparity are particularly apparent in cool temperate regions such as New Zealand. Viviparity enables the fetuses to be protected inside a "mobile incubator". Furthermore, development would be accelerated by the thermophily of the mother. Embryos that are developing rapidly (due to the thermal preferences of the mother) would have a high rate of oxygen consumption. The high degree of placentation possessed by the species (Barwick, 1959) would certainly facilitate the exchange of water, gases and

wastes. In fact, this would seem to be placentation's most significant function in this case; it is unlikely that it contributes in a major way to the nutrition of the embryos, since the yolks are of a substantial size.

Population dynamics

Several hundred skinks were collected from Kaitorete Spit, but since they were to serve as subjects for a physiological study, the larger individuals were usually selected for capture. As a result, the weight and length data on lizards from the spit cannot be used to establish the age structure of the population. Field observations at the spit suggest, however, that if the young survive the first growing season and the winter, the chances are good that they will grow to maturity. From Barwick's (1959) and personal observations, it was determined that the lizards collected at the spit would have had at least two growing seasons (i.e. they would be at least 15 months old) and that most would have had three to four growing seasons. Considering that growth is very slow after sexual maturity (Barwick, 1959; personal observations), skinks which were greater than 6 cm SVL would probably be more than four years old. Since individuals up to 7.7 cm SVL were collected, some individuals in the population must have been very old indeed. Compared to the small lizards, L. laterale (Tinkle, 1967) and U. stansburiana (Brooks, 1967), L. zelandica is long-lived.

A fortuitous collection made in a relatively isolated marsh area (see Study areas) does provide some information on population structure. The size and weight distribution of this

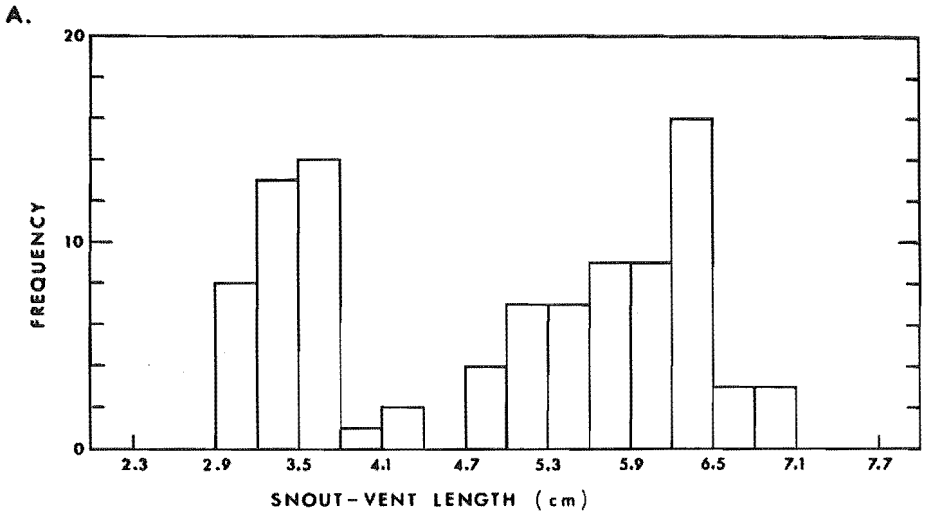
small population is illustrated in Fig. 3. These data were obtained from a large aggregation of skinks which were found on a cool, sunny winter morning (July 1, 1972). Although some other cover is available in this area, the bulk of the population apparently occurred in or under one rotten log. Since the area is occasionally inundated with water, it is probable that this log provided the most suitable cover in the area. A total of 96 skinks, most of them relatively torpid, were extricated from the log. They tended to be aggregated into groups of 4-10 individuals in pockets of decayed wood. The individuals with a SVL of less than 4.7 cm and weighing less than about 1.5 g would include those which were approximately 6 months old (if born in late December) and 18 months old. As is clear from Fig. 3, these two year classes cannot be distinguished. The differences in birth dates and weights, and in growth rates among individuals undoubtedly account for much of this overlap. The explanation for the hiatus in the distribution between the juveniles and the older adults is not clear. It may reflect poor reproductive success or high mortality of the young in these years. Alternatively, many individuals within the second and third year classes may have dispersed or been driven away from the log and become scattered throughout the marsh area using cover which was less accessible for collection. Although little is known about the intraspecific behaviour of scincids because of their retiring habits, there is no evidence for territoriality or for any form of aggressive behaviour in L. zelandica (Barwick, 1959; personal observations). Dispersion of juveniles and young adults from the log, however, cannot be ruled out as a possibility.

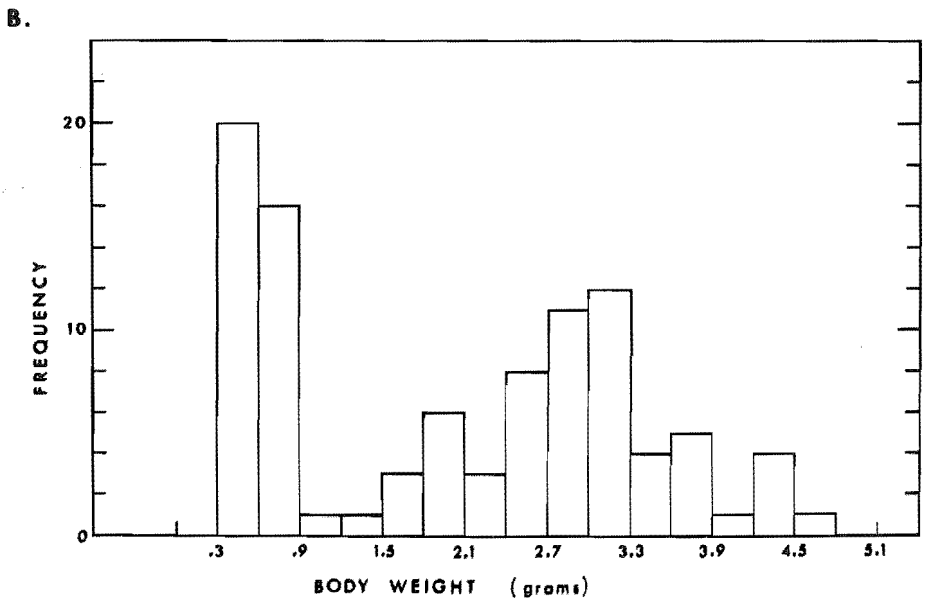
Fig. 3: Histograms.

A. Size distribution

B. Weight distribution

Data were obtained from 96 skinks (L. zelandica) which were collected in or under one rotten log situated in a damp, salt-marsh area on the New Brighton Spit (see Fig. 1).
See text for details of collection.


A.
20
10
0
FREQUENCY
2.3
2.9
3.5
4.1
4.7
5.3
5.9
6.5
7.1
7.7
SNOUT-VENT LENGTH (cm)


B.
20
10
0
FREQUENCY
.3
.9
1.5
2.1
2.7
3.3
3.9
4.5
5.1
BODY WEIGHT (grams)

Turnover in the populations in the study areas cannot be determined with accuracy, but from observations made on growth and age distribution, four to five years would not seem unrealistic. Female L. zelandica do not contribute to the population until they are slightly more than three years old and the chances are probably good that they would participate in one or two more breeding seasons. For some populations of U. stansburiana, turnover is almost annual (Tinkle, 1967), whereas for L. laterale, individuals which survive the first year have a good chance of taking part in at least one other breeding season (Brooks, 1967).

The availability of food and cover are probably the most important factors which determine the population density of skinks. At Motunau Island, in contrast to the other two study areas, lizards (three species) occur in high density (personal observation). The presence of colonial sea birds on the island probably has the effect of increasing the invertebrate fauna which is necessary to sustain the high lizard density. This situation has also been reported for other small islands off the east coast of New Zealand (see Whitaker, 1968). As described above, more cover is available for the lizards on Motunau Island than at either the marsh area or the spit.

During the thermophysiological study, a large number of skinks were dissected. The only endoparasite found was a nematode in the cloaca of one skink. The female nematode has been identified as Pharyngodon sp. (Family Oxyuridae). The nematode is apparently similar to P. mudgi (see Specian and Ubelaker, 1974) found in Coleonyx brevis (Gekkonidae) from West Texas, although a male would be required for species identification (Dr W.C. Clark, pers. comm.). Barwick (1959) reported

that over a third of the individuals examined harboured Pharyngodon sp. It is not clear how the nematode could exist at the spit in such low densities (a fraction of one per cent). Because of the activity and the relatively large size (more than 4 mm) of this nematode, it would be difficult to overlook in dissections of fresh material. Brooks (1967) reported that tapeworms, nematodes and flukes occurred in the intestines of 53, 51 and 27% respectively of the L. laterale examined.

None of the skinks collected at the Avon-Heathcote Estuary and at Kaitorete Spit had mites, although high infestations occur at Motunau Island and other islands (see Whitaker, 1968).

Most of the individuals captured at the spit had their original tails, whereas at Motunau Island almost all the lizards captured had lost some part of their tail. Only 21% of the individuals in the marsh area had regenerated tails. In Barwick's population, the incidence was about 67%. Lost toes were not uncommon for lizards at the spit and three individuals had lost a front limb.

Thermal relations

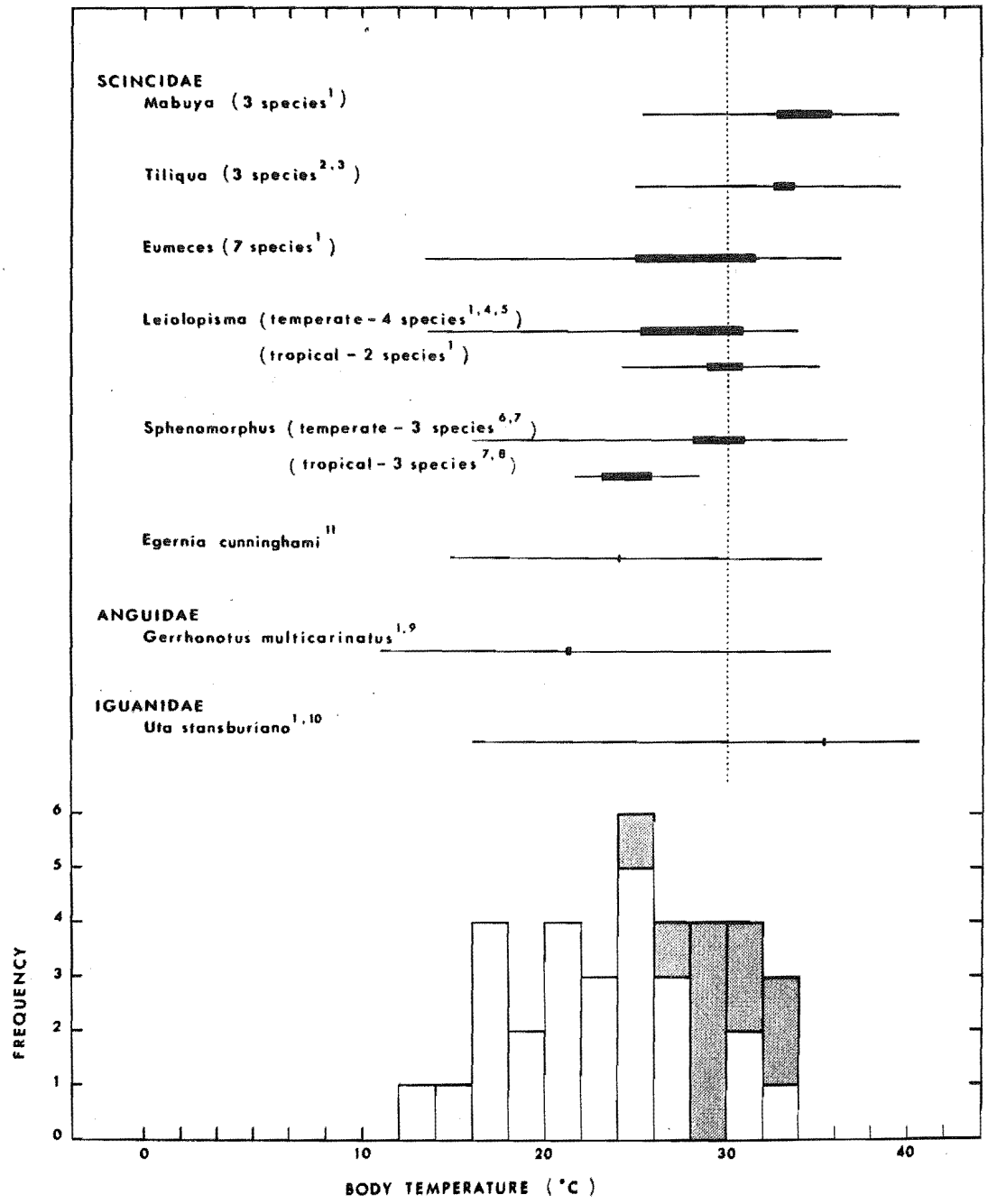
Because of the small size, covertness and celerity of L. zelandica, it is difficult to characterise its thermal relations on the basis of body temperatures (see Brattstrom, 1965; Templeton, 1970). Some body temperatures were obtained, however, at Motunau Island and Kaitorete Spit (see Fig. 4). They ranged from 13.6° to 32.9°C (n = 35). Although all the measurements were taken when it appeared that most of the individuals in the population were active, there is

Fig. 4: Histogram of body temperatures of 35 L. zelandica.

The unshaded portion represent data obtained from Motunau Island and the shaded area, data obtained from Kaitorete Spit. For comparison, the thermal relations of those species of Scincidae for which data are available have been summarised. Two other lizards which are sympatric and relatively eurythermic, but which have very different thermal preferences, have also been included. The dotted vertical line indicates the body temperature of 30°C. Range of body temperatures is represented as a thin horizontal line and the range of mean body temperatures of different species, as a shaded rectangle.

References are: 1. Brattstrom, 1965 (cited in Table 7); 2. Licht et al., 1966; 3. Bartholomew et al., 1965; 4. Morris, 1971; 5. Present study; 6. Spellerberg, 1972; 7. Veron and Heatwole, 1970; 8. Alcala and Brown, 1966; 9. Cunningham, 1966; 10. Roberts, 1968; 11. Barwick and Bryant, 1966.

Note: Data from two Lygosoma spp. from North America (Brattstrom, 1965) have been included with those of other temperate-dwelling leiolopismids (see Greer, 1970 regarding the taxonomic status of these species).


SCINCIDAE
Mabuya (3 species[1])
Tiliqua (3 species[2,3])
Eumeces (7 species[1])
Leiolopisma (temperate - 4 species[1,4,5])
(tropical - 2 species[1])
Sphenomorphus (temperate - 3 species[6,7])
(tropical - 3 species[7,8])
Egernia cunninghami[11]
ANGUIDAE
Gerrhonotus multicarinatus[1,9]
IGUANIDAE
Uta stansburiana[1,10]
FREQUENCY
0
1
2
3
4
5
6
0
10
20
30
40
BODY TEMPERATURE (°C)

considerable variation in the body temperatures. Most of the data from the spit were obtained about a week before those from Motunau Island; all data were obtained under similar weather conditions. For the most part, the differences in the ranges of body temperatures from these two areas can be attributed to the methods of capture used and the thermal diversity in the habitats. The lizards at the spit were seized as they darted between the pingao when their cover was disturbed. At the island, however, the lizards were generally collected under rocks and debris since those in the dense vegetation and in front of burrows (see Study areas) were extremely difficult to capture.

Observations in the outdoor terrarium also indicate that L. zelandica is active over a wide range of temperatures. On one occasion, for example, when the cover was carefully lifted off a group of skinks in the terrarium at midnight during the winter (June 21), the skinks appeared relatively alert and moved to other cover. The substrate temperature was about 4°C. Laboratory experiments have indicated that some individuals are capable of voluntary locomotion at temperatures at least as low as -1°C. This temperature is slightly below the freezing point of their tissues which is about -0.7°C. At the other end of the biothermal range, L. zelandica was found to avoid body temperatures in excess of 33°C by burrowing. On one occasion in the terrarium, for example, when the substrate temperatures ranged between 39° and 56°C, most of the skinks were found aggregated under one piece of timber where the temperatures ranged between 29° and 33°C. The temperatures under other cover were generally greater than 35°C. No dead skinks were found in the terrarium during hot spells, attesting to their

well-developed temperature sense. On the basis of its thermal relations, L. zelandica can be described as eurythermic, i.e. active over a wide range of temperatures.

The term eurytherm has, unfortunately, been used to denote at least three different concepts in the literature. Brett (1956) and Cloudsley-Thompson (1970) have applied the term to fish and invertebrates (respectively) which tolerate acute or chronic exposure to a relatively wide range of temperatures. This use of the term would be inappropriate for lizards and other terrestrial vertebrate ectotherms (TVE) since a wide thermal tolerance is a general feature of TVE. This is not, of course, the case for aquatic ectotherms which generally live in relatively stable thermal environments (see Vernberg and Vernberg, 1970). Based on thermal tolerance, Dipsosaurus dorsalis would be classed as one of the most "eurythermic" lizards since it can tolerate temperatures from below the freezing point of its tissues, i.e. supercooled (Lowe, Lardner and Halpern, 1971), to about 48°C (Brattstrom, 1965). And yet, according to Brattstrom (1965), this species normally has a body temperature greater than 27°C when abroad.

Cowles (1962) uses the term, "eurytherm", to describe ectotherms which, unlike the more heliothermic species, have no temperature optimum and which appear to be relatively passive with regard to behavioural thermoadjustment. Using this approach, non-heliothermic, nocturnal and burrowing lizards could be considered as eurythermic. Some tropical anoles, in fact, have been described as eurythermic, using Cowles' definition (see Ruibal, 1961; Ruibal and Philibosian, 1970; McManus and Nellis, 1973). These lizards might be more realistically described as thermoconformers. Vernberg and

Vernberg (1970) and Morris (1971) have defined a eurytherm as an organism which is active in the field over a wide range of body temperatures. This does not imply that such an organism is incapable of behavioural thermoadjustment (see Heath, 1964). L. zelandica is conspicuously heliothermic when substrate temperatures are low. Once an equable temperature has been reached, this skink may utilise microclimates in the vegetation or it may become relatively indifferent to temperature changes within a certain range (see Lee and Badham, 1963). The small size of L. zelandica precludes significant physiological contributions to their thermoadjustment repertoire (see Morris, 1971). Small ectotherms heat and cool very rapidly because of their large surface area to mass ratio. Consequently, the body temperature of small non-heliothermic lizards would tend to be at the temperature of the substrate-air interface where they were found. Small size is, nevertheless, clearly advantageous for thermophiles in relatively cool habitats such as occur at high latitudes and altitudes - it permits them to elevate their body temperature rapidly by basking. But under conditions of high solar flux (intense insolation), a highly-developed temperature sense would be mandatory for a small thermophile, or overheating could occur.

L. zelandica is generally active on the sand dunes at the spit on any fine day throughout the year. In the winter, however, they are more active (hence, more readily collected) in the afternoon, whereas in the summer, they tend to be crepuscular. When substrate temperatures are above the skinks' maximum voluntary temperature (which, at the spit, can be most of the day during summer), very few skinks are seen in the vegetation or darting amongst the pingao. They have, for the

most part, burrowed under the plants. On winter days, the skinks can, by extensive basking, attain body temperatures similar to those achieved in the summer. A few body temperatures were obtained at the spit in the winter (July 2) at about mid-day. They ranged from 24.5 to 28.7°C (n = 4), even though the substrate temperature in the shade was about 12°C. Because behavioural thermoadjustment enables the skinks to raise their body temperatures in winter to within the normal activity range for summer, acclimatisation would not be expected to occur, to any great extent.

L. laterale is similar to L. zelandica in these respects. It, too, is active on days when the shade temperature is as low as 12°C, and it tends to be active in the afternoon in winter and crepuscular in summer (Brooks, 1967). Although L. laterale is more thermophilic than L. zelandica, they appear to be similar in their use of insolation and suitable microclimates to adjust their body temperatures. On these bases, L. zelandica, and perhaps other scincids, can be described as opportunistic or facultative thermophiles.

Observations in the winter at the marsh area (see above) suggest that under certain circumstances L. zelandica may remain inactive for days, perhaps even for weeks during cold weather. Most of the individuals collected from the end of the log, which was more exposed to direct insolation, had clearly been basking prior to capture. The temperatures of the torpid skinks were only a few degrees above 0°C, but these lizards were capable of locomotion when collected. After basking in the transparent collecting vessel, the skinks that had been torpid became alert and active. It is, thus, most unlikely that these skinks were brumating (see Mayhew, 1965). Brumation

is winter dormancy; it is a state which differs radically from simple cold torpor, in that it is preceded by lethargy which is triggered by changes in photoperiod (rather than in temperature *per se*).

In spite of the sparseness of the vegetation at the spit, there is a wide range of microclimates accessible to the lizards. For example, when the substrate near the pingao was 38°C at 1000 hours (November 2), temperatures within the plants ranged from 23.4° to 30.9°C. At the same time of the day in the winter (July 3), however, the substrate on the sunny side of the plant was about 17°C, while the substrate on the shady side still had frost on it. The temperatures within the vegetation ranged from about 3° to 9°C. Most of the lizards captured at the spit during the winter were warm to the touch and they had undoubtedly been basking.

The range and means of body temperatures for several scincid genera are presented in Fig. 4. A eurythermic anguid and a relatively eurythermic iguanid which are sympatric, but which have very different thermal preferences are included in Fig. 4 for comparison. The scincid genera have been arranged approximately in the order of their thermophily. The two more thermophilic genera of scincids (*Tiliqua* and *Mabuya*) appear to be relatively stenothermic, whereas the temperate-dwelling representatives of the genera *Eumeces*, *Leiolopisma*, *Sphenomorphus* and *Egernia* are more eurythermic. The less thermophilic tropical scincids (see Fig. 4) tend to be stenothermic due, to some extent, to the lack of lower temperatures in their environment. It would appear, however, that tropical sphenomorphids are physiologically adapted to be active at lower body temperatures than either their temperate-

dwelling congeners (see Veron and Heatwole, 1970) or the sympatric skink Mabuya rudis (Inger, 1959). The tropical sphenomorphids which have been studied are apparently obligative non-heliotherms and are very sensitive to overheating if restrained in the open and exposed to solar radiation. Alcala and Brown (1966) reported that the critical maximum body temperature of tropical sphenomorphids ranged between 35° and 37°C. The tropical leiolopismids, on the other hand, appear to be similar to their temperate-dwelling congeners in their thermal preferences. The anguid, Gerrhonotus multicarinatus, like many scincids, lives under the litter. While this anguid is generally thigmothermic, it is also known to bask under conditions of low ambient temperatures (Cunningham, 1966). It would seem, however, that this anguid is not as thermophilic as most scincids. Like L. zelandica, G. multicarinatus is active during the warmer portions of the day in the winter, but it tends to be crepuscular during the summer. U. stansburiana is considerably more thermophilic than L. zelandica and other leiolopismids, but it is very sluggish in its movements below 20°C (i.e. cold sensitive). Some utas are active in the winter on clear days and, like L. zelandica, they are most active in the afternoon during the winter and also tend to be crepuscular in the summer (Alexander and Brown, 1968).

Specific differences in thermal preferences are probably, for the most part, genetically-determined physiological adaptations to particular habitats rather than effects of acclimatisation or of environmental thermal diversity (Cloudsley-Thompson, 1971). By being eurythermic, small ectotherms like L. zelandica can remain active in suboptimal

thermal environments.

SUMMARY

1. Field observations of L. zelandica were made at three areas: Motunau Island; a salt marsh on New Brighton Spit; and Kaitorete Spit. These areas differ markedly in the availability and type of cover.

2. Lizards captured from the latter two areas were kept in an outdoor terrarium where further observations were made.

3. L. zelandica is typically alert, covert and celeritous when active; these factors may account for some of the success of this small lizard.

4. This skink is slow growing, slow to mature sexually and is relatively long-lived. Females do not contribute offspring to the population until they are about three years old.

5. Copulation occurs in the autumn, whereas females ovulate in the spring; this indicates that sperm storage must occur in this species.

6. Viviparity has undoubtedly facilitated dispersion into cooler habitats at high latitudes and altitudes.

7. Large aggregations of L. zelandica do occur in the field.

8. Turnover in the population would probably occur in four or five years.

9. Population density of this skink is determined by the availability of food and cover.

10. The only endoparasite found in L. zelandica from the spit was a nematode (Pharyngodon sp.).

11. Ectoparasitic mites tend to occur in lizards in island populations.

12. The incidence of caudal autotomy varies considerably between populations.

13. Body temperatures of L. zelandica obtained under suitable macroclimatic conditions ranged between 13.6° and 32.9°C (n = 35).

14. L. zelandica is eurythermic, but is conspicuously heliothermic when substrate temperatures are low. The term eurytherm is defined.

15. On the basis of its thermal relations, this skink can be described as an opportunistic or facultative thermophile.

16. L. zelandica appears to have a well-developed temperature sense and can avoid overheating in spite of its small size and rapid heat exchanges.

17. This skink is active on any clear, sunny day at all times of the year. Under certain circumstances L. zelandica may be inactive during a portion of the winter but such inactivity appears to be cold torpor.

18. L. zelandica is compared to other lizards (particularly scincids) with regard to thermal relations.

LITERATURE CITED

Alcala, A.C. and Brown, W.C. 1966. Thermal relations of two tropical lizards on Negros Island, Philippine Islands. Copeia, 1966: 593-594.

Alexander, C.E. and Whitford, W.G. 1968. Energy requirements of Uta stansburiana. Copeia, 1968: 678-683.

Bartholomew, G.A., Tucker, V.A. and Lee, A. 1965. Oxygen consumption, thermal conductance and heart rate in the Australian skink Tiliqua scincoides. Copeia, 1965: 169-173.

Barwick, R.E. 1959. The life history of the common New Zealand skink Leiolopisma zelandica (Gray, 1843). Trans. Roy. Soc. N.Z., 86: 331-380.

Barwick, R.E. and Bryant, C. 1966. Physiological and biochemical aspects of hibernation in the scincid lizard Egernia cunninghami (Gray, 1832). Physiol. Zool., 39: 1-29.

Brattstrom, B.H. 1965. Body temperatures of reptiles. Am. Midland Nat., 73: 376-422.

Brett, J.R. 1956. Some principles in the thermal requirements of fishes. Quart. Rev. Biol., 31: 75-87.

Brooks, G.R. 1967. Population ecology of the ground skink Lygosoma laterale (Say). Ecol. Monogr., 37: 71-87.

Burton, M.P. ed. 1967. Motunau Island, Canterbury, New Zealand. N.Z. Dept Sci. Industr. Res. Bull., 178.

Cloudsley-Thompson, J.L. 1970. Terrestrial invertebrates. In Comparative physiology of thermoregulation. Vol. I. Invertebrates and nonmammalian vertebrates. (Whittow, G.C. ed.) Academic Press, New York. 167-218.

Cloudsley-Thompson, J.L. 1971. The temperature and water relations of reptiles. Merrow, London.

Cowles, R.B. 1962. Semantics in biothermal studies. Science, 135: 670.

Cunningham, J.D. 1966. Thermal relations of the alligator lizard Gerrhonotus multicarinatus webbi. Herpetologica, 22: 1-7.

Fitch, H.S. 1954. Life history and ecology of the five-lined skink, Eumeces fasciatus. Univ. Kansas Publ., Mus. Nat. Misc. Publ., 8: 1-156.

Fitch, H.S. 1967. Ecological studies of lizards on the University of Kansas Natural History Reservation. In Lizard ecology : a symposium (Milstead, W.W. ed.) University of Missouri Press, Columbia, Mo. 30-44.

Greer, A.E. 1970. A subfamilial classification of scincid lizards. Bull. Mus. Comp. Zool., 139: 151-184.

Heath, J.E. 1964. Reptilian thermoregulation : evaluation of field studies. Science, 146: 784-785.

Inger, R.F. 1959. Temperature responses and ecological relations of two Bornean lizards. Ecology, 40: 127-139.

Lee, A.K. and Badham, J.A. 1963. Body temperature, activity and behaviour of the agamid lizard, Amphibolurus barbatus. Copeia, 1963: 387-394.

Licht, P., Dawson, W.R., Shoemaker, J.H. and Main, A.R. 1966. Observations on the thermal relations of Western Australian lizards. Copeia, 1966: 97-110.

Lowe, C.H., Lardner, P.J. and Halpern, E.A. 1971. Supercooling in reptiles and other vertebrates. Comp. Biochem. Physiol., 39A: 125-135.

Mayhew, W.W. 1965. Hibernation in the horned lizard Phrynosoma m'calli. Comp. Biochem. Physiol., 16: 103-120.

McCann, C. 1955. The lizards of New Zealand. Gekkonidae and Scincidae. Dominion Museum Bull. 17: 1-127.

McManus, J.J. and Nellis, D.W. 1973. Temperature and metabolism of a tropical lizard, Anolis acutus. Comp. Biochem. Physiol., 45A: 403-410.

Morris, R.W. 1971. On some aspects of the biology, thermal relations and thermophysiology of Leiolopisma lineoocellatum. Unpublished M.Sc. thesis, University of Canterbury, New Zealand.

Roberts, L.A. 1968. Oxygen consumption in the lizard Uta stansburiana. Ecology, 49: 809-819.

Ruibal, R. 1961. Thermal relations of five species of tropical lizards. Evolution, 15: 98-111.

Ruibal, R. and Philibosian, R. 1970. Eurythermy and niche expansion in lizards. Copeia, 1970: 645-653.

Specian, R.D. and Ubelaker, J.E. 1974. Two species of Pharyngodon Diesing, 1861 (Nematoda:Oxyuridae) from lizards in West Texas. Proc. Helminthological Soc. Wash., 41: 46-51.

Spellerberg, I.F. 1972. Thermal ecology of allopatric lizards (Sphenomorphus) in southeast Australia. II. Physiological aspects of thermoregulation. Oecologia (Berl.), 9: 385-398.

Templeton, J.R. 1970. Reptiles. In Comparative physiology of thermoregulation. Vol. I. Invertebrates and non-mammalian vertebrates (Whittow, G.C. ed.) Academic Press, New York. 167-218.

Tinkle, D.W. 1967. Home range, density, dynamics and structure of a Texas population of the lizard Uta stansburiana. In Lizard ecology: a symposium (Milstead, W.W. ed.) University of Missouri Press, Columbia, Mo. 5-29.

Vernberg, F.S. and Vernberg, W.B. 1970. The animal and the environment. Holt, Rinehart and Winston, New York.

Veron, J. and Heatwole, H. 1970. Temperature relations of the water skink, Sphenomorphus quoyi. J. Herpetology, 4: 141-153.

Whitaker, A.H. 1968. Lizards of the Poor Knights Islands. N.Z. J. Sci., 11: 623-651.

CONTENTS

PART B Some aspects of the behaviour and physiology of *Leiolopisma zelandica* at temperature extremes

LIST OF FIGURES

PART B

PART B

SOME ASPECTS OF THE BEHAVIOUR AND PHYSIOLOGY OF LEIOLOPISMA ZELANDICA AT TEMPERATURE EXTREMES

INTRODUCTION

During a study of the thermophysiology of L. zelandica over a wide temperature range, experiments were conducted at body temperatures below the freezing point of its tissues (supercooling experiments) and above its maximum voluntary temperature (high temperature experiments). The purpose of this report is to describe certain responses of this skink to these temperature extremes in greater detail than in Section 1.

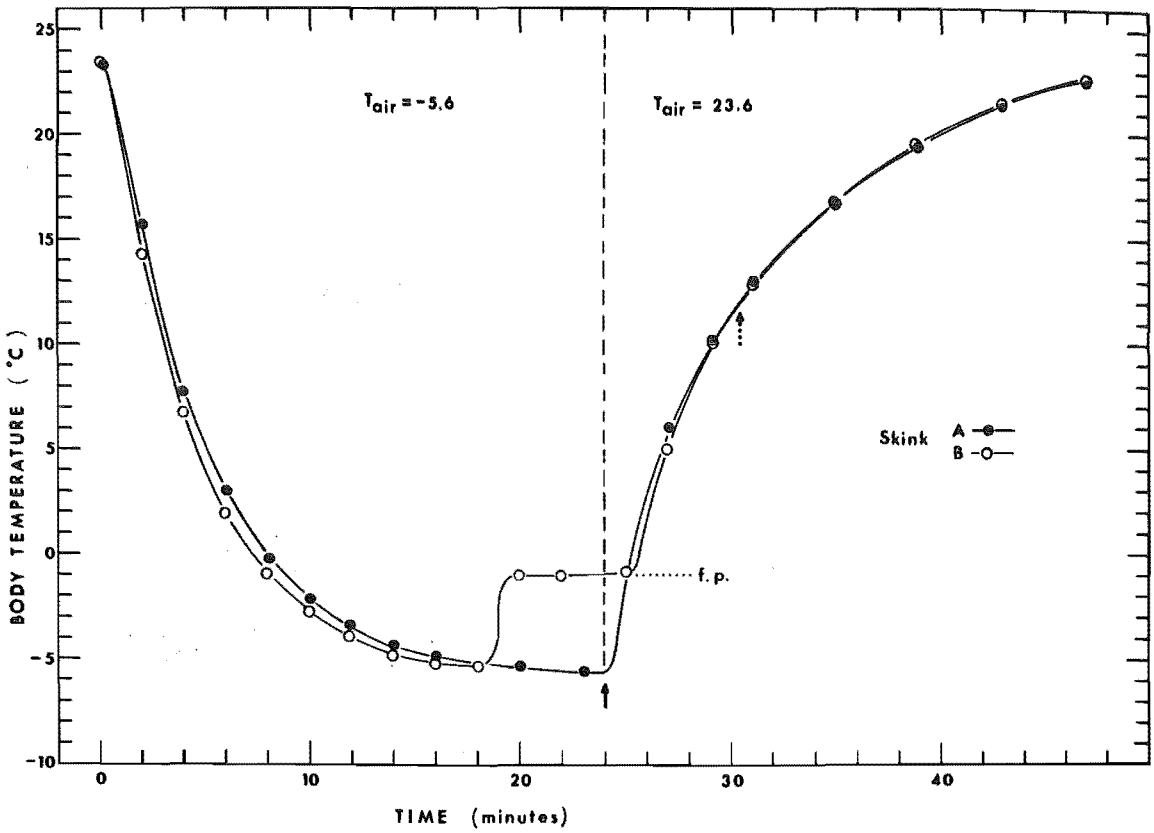
SUPERCOOLING EXPERIMENTS

General procedures and observations

Two procedures were used to supercool the lizards to about -5°C; either they were cooled rapidly by being placed into a temperature cabinet which was at about -5°C (see Fig. 1), or they were cooled rapidly to 0°C then slowly supercooled. The subjects generally tolerated either procedure. On one occasion, however, when two subjects were rapidly cooled to -5°C, no cardiac activity (ECG) could be detected from one of the subjects. But cardiac activity did resume when this subject was rewarmed. This suggests that thermal shock may have occurred, although this effect did not recur when it was

Fig. 1: Rapid supercooling, spontaneous crystallisation and rewarming curves.

Solid-line arrow indicates when subjects were removed from the cabinet, dashed vertical line separates the cooling and warming curves, dotted horizontal line indicates the freezing point (f.p.) of the tissues of Skink B (female with large ovarian yolks) and dotted-line arrow indicates when Skink A (male) first appeared to ventilate. Skink B was killed by the effects of spontaneous crystallisation.


T_{air} = -5.6
T_{air} = 23.6
Skink A
B
f. p.
BODY TEMPERATURE (°C)
TIME (minutes)
25
20
15
10
5
0
-5
-10
0
10
20
30
40

immediately supercooled a second time.

During slow supercooling, ventilation was observed at body temperatures as low as -1°C and the cardiac rate decreased markedly at about -2°C. Some unrestrained subjects were capable of voluntary locomotion to at least -1°C, although they were not necessarily able to right themselves when placed on their backs. They were incapable of locomotory activity at -2°C, even when prodded.

Summer- and winter-acclimatised skinks supercooled equally well. It appeared, however that females with large ovarian yolks (during winter and spring) were more susceptible to spontaneous crystallisation (see Fig. 1). Most subjects, male or female, which spontaneously crystallised during experiments, did so after they had been at -5°C or lower for several minutes. When individuals were slowly supercooled below -5°C they invariably crystallised near -6°C. This appears to be the supercooling limit of this species (see Lowe, Lardner and Halpern, 1971; Spellerberg, 1972). One individual, however, did remain supercooled at about -6°C overnight, but failed to revive when rewarmed, although cardiac activity did resume. Since the skeletal muscles and peripheral nerves responded to electrical stimulation, it would seem that the central nervous system had been damaged by the prolonged exposure to such low temperatures. It is most likely that the damage was due to a combination of the effects of anoxia and disruption of central synaptic membranes.

Only two subjects failed to become supercooled when placed into the cabinet at -5°C. Because of the latent heat of crystallisation, their body temperature remained at the freezing point of their tissues. When rewarmed after several

minutes at this temperature, both subjects recovered their normal functions.

Of the subjects which were supercooled to -5°C, some were maintained in that condition for only a few hours (i.e. "acute" supercooling) to determine cardiac activity. Other subjects were either supercooled for periods of 6-8 hours to determine oxygen consumption or for up to 15 hours to investigate the effects of "chronic" supercooling.

Acute supercooling

All the subjects which supercooled during acute supercooling experiments were alert immediately upon warming. Some began to ventilate while still slightly supercooled (as low as -1.3°C), while others had warmed to about 12°C before ventilation resumed (see Fig. 1). Lizards which were supercooled to -5°C, rewarmed, then supercooled again (repetitious acute supercooling) survived the treatment. They, like other subjects, were returned to the terrarium for subsequent observation. Because all the subjects had been toe-clipped, they could be reidentified. Up to six months later, they were healthy and alert. None of the subjects recovered after supercooling experiments only to die hours or days later. This would indicate that acute and repetitious supercooling is not harmful. Halpern and Lowe (1968) have reported a similar finding for Uta stansburiana.

Chronic supercooling

Of the thirty skinks which were used for oxygen consumption determinations, only fifteen were still supercooled at -5°C after 6-8 hours. All but one of the survivors recovered immediately upon warming. One subject took about

20 minutes to recover. The behaviour of this subject, however, appeared to be normal two months after the experiment. Upon rewarming, the hearts of the non-survivors began beating normally, but because ventilation did not resume, the cardiac rate soon decreased due to anoxia.

When cardiac activity was monitored during chronic supercooling, several changes were found to occur in their ECG. The amplitude and the frequency of the electrocardiac signals decreased gradually during the first 4-6 hour period. Subjects which were supercooled for 12-15 hours had no detectable cardiac activity. When warmed to about $0^{o}C$, small RS potentials (from ventricular depolarisation) became evident in their ECG. The amplitude and frequency of these signals increased rapidly during warming. The cardiac rate at about $20^{o}C$ for these subjects was normal, but the P-R interval was longer than normal and sometimes a T_a wave (i.e. T wave of the auricles) occurred prior to the RS wave. This indicates first degree AV block; it may have been due to the effects of anoxia or to the effects of ionic changes on conduction across the auriculo-ventricular junction. Unusually large T waves also occurred and may reflect changes in the vector of ventricular repolarisation and/or extracellular hyperkalemia (see Hoffman and Cranefield, 1960).

A few subjects which had been supercooled overnight at $-5^{o}C$ were subjected to acute supercooling after having been rewarmed. On the second warming, only P waves were initially present, indicating complete AV block. This was followed by partial AV block and finally first degree block after they were warmed to $20^{o}C$. Not all subjects survived this procedure since some spontaneously crystallised, but those which did, were

still alive and healthy months later.

Spontaneous crystallisation

As is illustrated in Fig. 1, the result of freezing from the supercooled state is a rapid increase in the body temperature to the freezing point of the tissues (-0.7°C). This increase is due to the latent heat of crystallisation. Subjects do not revive after spontaneous crystallisation, even if they are immediately rescued from the cabinet. Monitoring the ECG during spontaneous crystallisation revealed that the cardiac rate increases commensurate with the temperature at the freezing point of the skink's tissues. When the tail is pulled off such subjects, no caudal movements occur, whereas tails pulled off slightly supercooled skinks responded by slow rhythmical movements from side to side. These movements are comparable to those of non-supercooled, torpid skinks. Since it appears that tail contraction occurs as the result of injury potentials at the damaged end of the cord*, the lack of movement in tails of skinks that had spontaneously crystallised would indicate that the central nervous system had ceased to function.

When subjects which had frozen during oxygen consumption determinations at -5°C were warmed, their hearts resumed beating, their skeletal muscles were excitable by stimulation but their tails did not contract when pulled off. It would be

---

* If, for example, the spinal cord is severed between spinal nerves XIX and XX (which is about mid-way between the brachial and sacral nerves), tail contractions occur even though the tail is intact. No movement occurs, however, if the cord is cut anterior to XIX. When movements cease after cutting between XIX and XX, they can be initiated repeatedly by cutting the cord between posterior vertebrae. Tail movements become particularly vigorous, however, as the cord is cut posterior to the sacral region.

interesting to determine the nature of the damage to the central nervous system. It seems unlikely that the damage is due primarily to ice formation in the cells since some subjects in the temperature cabinet were removed moments after the temperature change began to occur and yet they did not recover. Microscopic crystals may, however, have been formed during the increase in temperature (i.e. spontaneous crystallisation).

The facility with which lizards and other reptiles supercool has been attributed to the presence of scales which are relatively impermeable to water and which, most importantly, prevent seeding of ice crystals (Halpern and Lowe, 1968). Spontaneous crystallisation is, however, a stochastic event hence not entirely predictable. On one notable occasion, the tail was pulled off a subject after acute supercooling and the subject was placed back into the cabinet. Four hours later, the subject was still supercooled in spite of the exposed tissues. Part of the success of the supercooling experiments in this study may be attributed to the effect of the very cold cooling coils in the cabinet (see Appendix C). At such low temperatures, the coils would have taken much of the water vapour out of the air.

HIGH TEMPERATURE EXPERIMENTS

General observations

The highest body temperature recorded for skinks captured in the field was 33°C. Preliminary tests indicated that L. zelandica could tolerate body temperatures as high as 40°C for 15-20 minutes and 41°C for a few minutes. The procedure used in these experiments was to heat the lizards

slowly to 35°C and then rapidly to 40°C. When cardiac and ventilatory activity were being monitored, the skinks were taped on to a stretcher. Restrained subjects generally remained inactive even at 41°C. The behaviour of lizards which were left free to move about a container in the temperature cabinet was not consistent. Some individuals were inactive, while others darted at the walls of the container, becoming quite unco-ordinated and, as a result, often falling on their backs. If they were unable to right themselves and were not rescued immediately, they did not recover. Both the active and inactive lizards (at 40°C) died without spasms or other visible signs of heat damage (see Licht, Dawson and Shoemaker, 1966). Tails pulled off dead subjects which had been removed from the cabinet contracted vigorously; hearts continued to beat; skeletal muscles responded to electrical stimulation, but ventilation had ceased and the animals were limp. As with low temperature damage, the central nervous system is implicated, but in this case it would seem that the brain is more thermolabile than the spinal cord.

Unlike the situation for supercooling, acclimatisation appears to be an important factor in heat resistance. Six attempts were made to heat skinks to 40°C in the winter; all developed ECG irregularities at about 38°C and one died during the experiment. Consequently, the observations described in this report refer to summer-acclimatised animals.

Cardiac activity

A number of interesting ECG features developed between 40°C and 41°C (see Fig. 2). ECG at 40°C had P-R intervals that were longer than would be expected on the basis of

Fig. 2: Electrocardiograms.

a. Records before and after third degree SA block and first degree AV block; cardiac rate decreased from 206.6 to 81.6 beats/minute. T_a is present. Final record obtained during cooling, rate is 153.9 beats/minute.

b. Record illustrating large RS waves of premature electrocardiac cycles followed by normal T-P intervals.

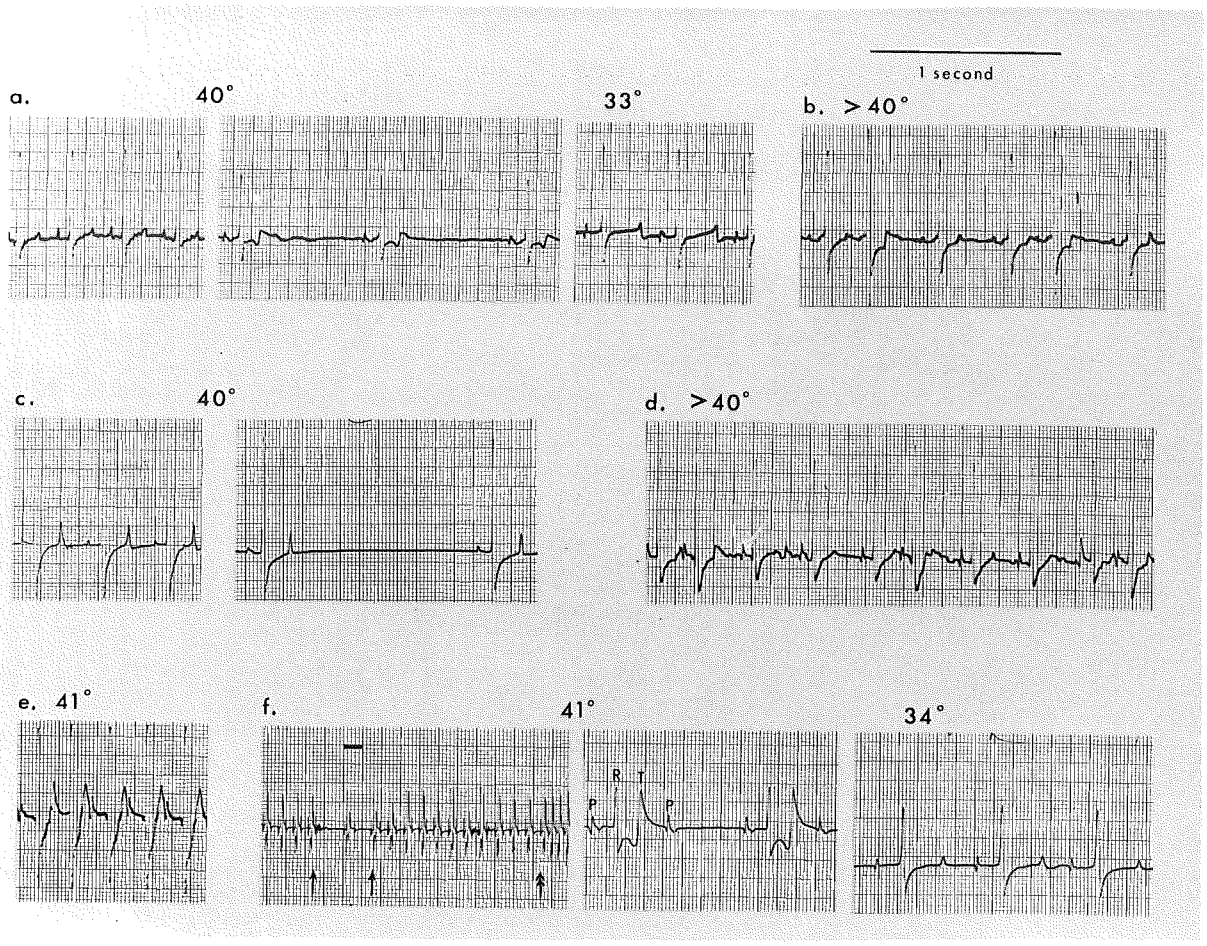
c. Records before and after third degree block and slight first degree AV block; rate decreased from 176.5 to 49.3 beats/minute.

d. Record similar to (b) except three normal cycles between each premature cycle.

e. Record illustrating highest rate obtained, (297 beats/minute) and first degree AV block. Subject died during experiment.

f. Records illustrating third degree SA block (between single arrows on low speed record); first degree and 2:1 (second degree) AV block (between second single and the double arrow on low speed record) and only first degree AV block (after double arrow). Second record, 2:1 block at faster paper speed; note unusual T wave. Final record obtained during cooling, rate is 117.6 beats/minute.

Horizontal bar in right hand corner is applicable to all records except low speed record in (f). Short bar in (f) is also one second.


1 second
a.
40°
33°
b. >40°
c.
40°
d. >40°
e. 41°
f.
41°
34°
R
T
P
P

a) the trend from 35° to 40°C, b) the corresponding ventricular activity at that temperature and c) the presence of a T_a wave in the P-R interval. These factors suggest that first degree AV block had occurred. Two of the sixteen subjects also developed SA block (Fig. 2 a and c). At temperatures greater than 40°C, unusually large RS waves of premature electrocardiac cycles occurred (Fig. 2 b and d). The T-P interval (from the end of the T wave to the beginning of the P wave), which follows the premature cycle, is of the same duration as for T-P intervals between normal cycles. One individual at 41°C developed partial SA block and first degree and 2:1 (second degree) AV block. All the individuals, except one which died during an experiment, were still alive the day after. The highest cardiac rate recorded for L. zelandica was 297 beats/minute but was taken from the subject which subsequently died. First degree AV block is evident in this subject's ECG (Fig. 2 e).

Ventilatory activity

At temperatures of 40°C or greater, the ventilatory rate was, for a short time, very rapid. The response was similar to that of the skink Eumeces obsoletus (Dawson, 1960). In spite of hyperpnoea, the animals were not panting as described by Templeton (1960) for Dipsosaurus dorsalis, nor were they gaping like Gerrhonotus multicarinatus (Dawson and Templeton, 1960). After only a few minutes at temperatures above 40°C, ventilation decreases markedly, becomes irregular and eventually ceases. If the skinks are not rescued immediately, they die. Damage to the respiratory centres of the brain probably accounts for the cessation of breathing. That death ensues so

rapidly after this event, would undoubtedly be due to anoxia, since the very high metabolic demands of the brain and other tissues at elevated temperatures could not be met.

SUMMARY

1. Experiments were conducted at both temperature extremes of L. zelandica; temperatures below the freezing point of its tissues (supercooling) and above its voluntary maximum.
2. Ventilation and voluntary locomotion occur as low as -1°C.
3. The ability to supercool is not affected by acclimatisation, but gravid females appear to be particularly susceptible to spontaneous crystallisation.
4. The supercooling limit for L. zelandica is near -6°C.
5. Several minutes of freezing without supercooling does not appear to be detrimental.
6. No unusual effects were noted in the ECG of this species after acute supercooling to 4 to 5 degrees (C) below the freezing point of its tissues (-0.7°C), whereas first degree AV block occurred after chronic supercooling. All supercooled subjects survived when warmed, except one which had been at -6°C.
7. This skink can tolerate repetitious acute supercooling and chronic followed by acute supercooling. In the former case, no unusual effects occurred. In the latter case, however, complete, partial and first degree AV block occurred during the rewarming process. Survivors did not appear to have been damaged by the treatment.

8. Spontaneous crystallisation killed subjects in spite of the fact that their hearts continued to beat and peripheral nerves and skeletal muscle remained excitable. Evidence suggests that it is the central nervous system (CNS) that is damaged.

9. In skinks warmed after several hours of freezing, the heart resumes activity and skeletal muscle remains excitable, but the CNS is damaged.

10. This skink tolerates body temperatures as high as 40°C for 15-20 minutes and 41°C for a few minutes.

11. In skinks cooled after heat death, hearts continued to beat, skeletal muscles, peripheral nerves and the spinal cord were excitable, but the brain was damaged.

12. Winter-acclimatised skinks do not tolerate 40°C.

13. First degree AV block and also, in two cases, SA block occurred at 40°C. Above 40°C, various ECG aberrations were noted including premature electrocardiac cycles, SA block and first and second degree AV block.

14. The skinks lack panting or gaping responses to high temperatures. Heat damage and cessation of breathing appear coincidentally and the resulting anoxia leads to heat death.

LITERATURE CITED

Dawson, W.R. 1960. Physiological responses to temperature in the lizard Eumeces obsoletus. Physiol. Zool., 33: 87-103.

Dawson, W.R. and Templeton, J.R. 1966. Physiological responses to temperature in the alligator lizard, Gerrhonotus multicarinatus. Ecology, 47: 759-765.

Halpern, E.A. and Lowe, C.H. 1968. Metabolism of the iguanid lizard Uta stansburiana in the supercooled state. Physiol. Zool., 41: 113-124.

Hoffman, B.F. and Cranefield, P.F. 1960. Electrophysiology of the heart. McGraw-Hill, New York.

Licht, P., Dawson, W.R. and Shoemaker, V.H. 1966. Heat resistance of some Australian lizards. Copeia, 1966: 162-169.

Lowe, C.H., Lardner, P.J. and Halpern, E.A. 1971. Supercooling in reptiles and other vertebrates. Comp. Biochem. Physiol., 39A: 125-135.

Spellerberg, I.F. 1972. Thermal ecology of allopatric lizards (Sphenomorphus) in southeast Australia. I. The environment and lizard critical temperatures. Oecologia (Berl.), 9: 371-383.

Templeton, J.R. 1960. Respiration and water loss at the higher temperatures in the desert iguana, Dipsosaurus dorsalis. Physiol. Zool., 33: 136-145.

APPENDICES

LIST OF FIGURES

APPENDIX A: OUTDOOR TERRARIUM

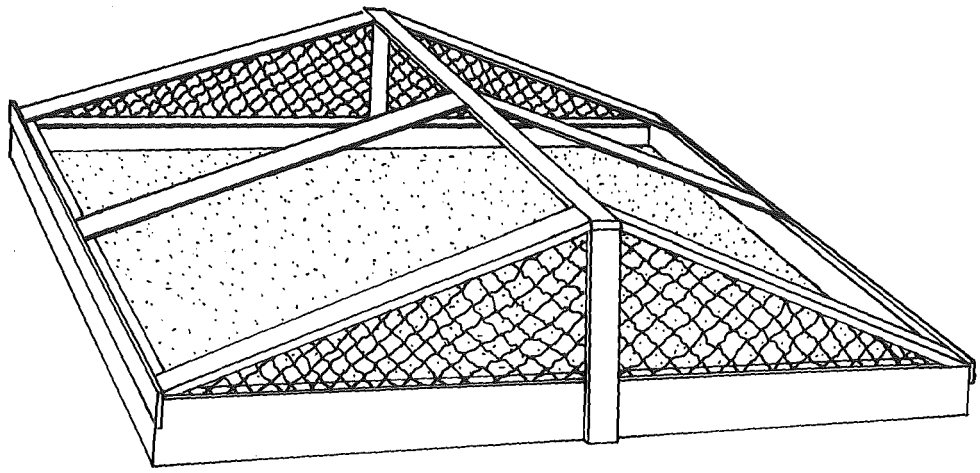
It was necessary to keep a large number of lizards under prevailing macroclimatological conditions for experimental use. For this purpose, an outdoor terrarium (9 m^2) was constructed on the roof of the Zoology Building of the University of Canterbury. Fig. 1A illustrates the general appearance of this structure. Potential bird predators such as the magpie (Gymnorhina hypoleuca) are kept out by wire netting. A ledge lined with galvanised iron prevents the lizards from escaping (see Fig. 1B). A natural habitat is simulated with sand (about 10 cm deep) and a variety of objects for cover including driftwood, vegetation, schist (rock) and timber. Water is provided in a tray. Except for the period when post-absorptive subjects were required for experiments, Tenebrio larvae were available in a feeding station. During hot periods in the summer, it is necessary to dampen the sand periodically to maintain a variety of microclimates under the cover.

Fig. 1: The outdoor terrarium.

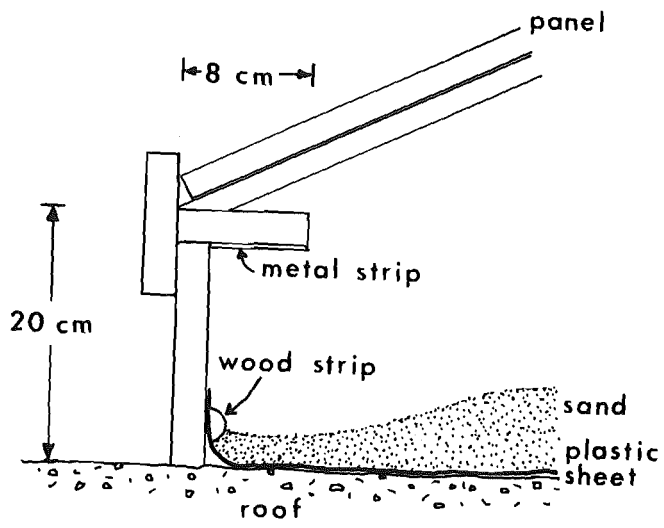
A. gives the general appearance of the 3 m by 3 m terrarium which was provided with wire-covered panels (not shown for clarity) to keep out potential bird predators.

B. is a cross-section of a portion of the structure, illustrating the features designed to make the lizard terrarium escape-proof.

A.



B.


panel
8 cm
metal strip
20 cm
wood strip
sand
plastic
sheet
roof

APPENDIX B: FLASK CONSTANTS FOR WARBURG MANOMETRY WITH WHOLE ANIMALS

The theoretical basis for the determination of oxygen consumption by Warburg manometry is outlined in detail for tissues and for lower levels of organisation in Umbreit et al. (1972). Certain modifications are required, however, to apply this method of respirometry to whole animals.

To obtain the oxygen consumed (x) in µl at STP (0°C and 760 mm of mercury), the observed change in fluid height (h) of the open side of the manometer is multiplied by the flask constant (k). Substituting for k:

$$x = hk = h\ (V_g \frac{273}{T} + V_f\ \alpha)\ (P_o)^{-1} \qquad (1)$$

degrees Kelvin; V_f is the fluid volume (of a brei); α is the solubility constant for oxygen; and P_o is the standard pressure (equivalent to 10^4 mm of Brodie's fluid).

The term $V_f\ \alpha$ in (1) cannot readily be determined for whole animals and, in any case, it would be negligible compared with V_g so that (1) can be simplified to:

$$x = h\ (0.0273\ V_g)\ (T)^{-1} \qquad (2)$$

where the constant 0.0273 is obtained by substitution of the value for P_o in (1). In this study, V_g was taken to be the calibrated volume of the flask-manometer unit minus the volume of the KOH and the skink. The volume of the units was determined by the mercury method (see Umbreit et al., 1972). The volume of each skink was determined after the experiment by placing the skink into a graduated cylinder and measuring the volume of water it displaced when totally submerged.

APPENDIX C: TEMPERATURE CABINET AND EQUILIBRATION EXPERIMENTS

To characterise some aspects of the thermophysiology and behaviour of small lizards at constant or equilibrated body temperatures, it was necessary to construct a temperature cabinet. Several design features were important. It was desirable that body temperatures be controlled to within 0.1°C over a wide temperature range. The operating range of the cabinet should extend at least over the biothermal range of terrestrial vertebrate ectotherms. The subjects in the cabinet should be relatively isolated from direct radiative sources and sinks, forced convection and motor vibrations. It was essential that relatively noise-free recordings of bio-electrical activity could be obtained from subjects inside the cabinet.

DESIGN AND OPERATION OF THE CABINET

An antiquated refrigerator cabinet (0.027 m^3) was obtained; it had been stripped inside and fitted with double Perspex windows. The cabinet was subsequently modified by the installation of a partition assembly, fan, heater, cooling system and a commercial temperature-controlling device.

Cut-away side and front views of the cabinet are shown diagrammatically in Fig. 2. The H-shaped partition (in Fig. 2B) is sealed at the back and bottom with weather stripping and enclosed with heavy plastic panels at the front to provide four compartments. The partitions facilitate in directing the air flow through the main compartment, across the cooling coils and heater wires and finally back into the main compartment. They also insulate the subjects in the main compartment from the direct effects of the heat sources and sinks. The fan motor vibrations are minimised in two ways. The platform on which the subjects are placed is suspended by wires and the fan motor is firmly mounted onto the partition assembly which rests on weather stripping.

One of the simplest methods of temperature control is

Fig. 2: Schematic diagram of the temperature cabinet.

A. Cut-away side view

B. Cut-away front view

Shaded arrows indicate the flow of air in the cabinet. Letters in the diagrams represent the following:

A. Gallenkamp Compenstat (TM 510).

B. Switches for motor of cooling system, fan and heater, and light.

C. Double perspex windows.

D. Panel for thermistor jacks.

E. Shield for cooling coils at back of cabinet.

F. Sensing bulb of Gallenkamp unit (A).

G. Metal pan containing experimental subjects (see Appendix D).

H. Asbestos platform suspended by wires.

J. Light.

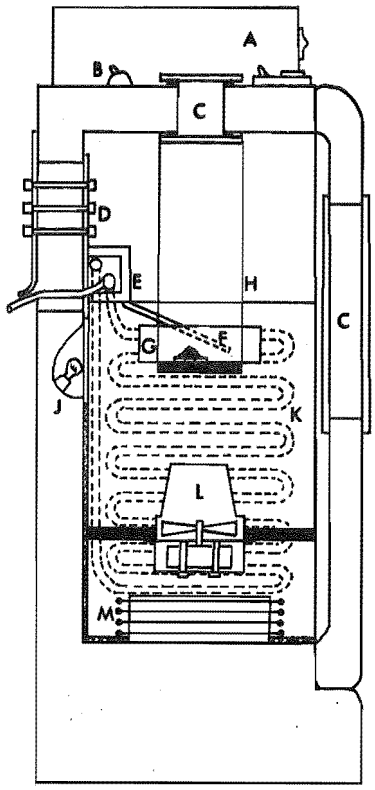
K. Cooling coils held with spacers and brackets.

L. Fan mounted on partition and surrounded by a vent.

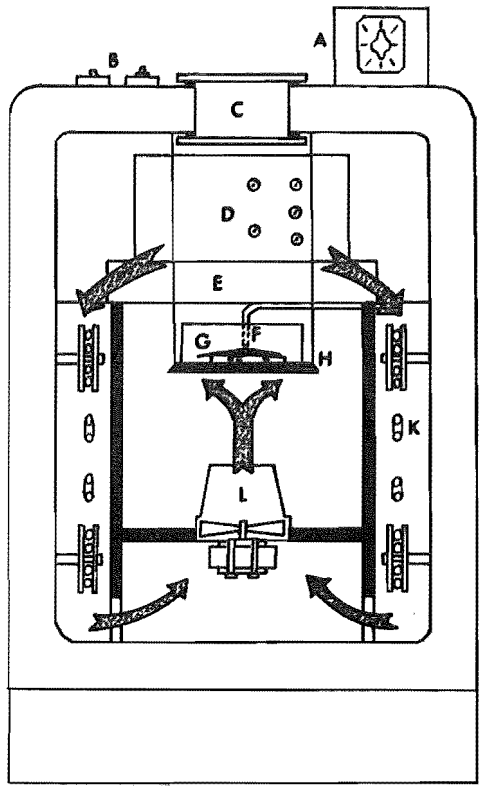
M. Heater wires strung across partition opening.

Fig. 3: Air temperature oscillations when the core temperature of a 1.4 g skink is constant at 0°C (see text for details).

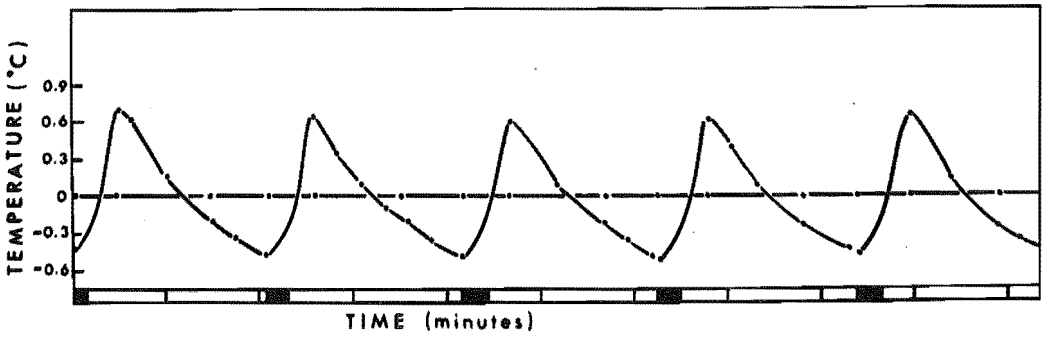
Shaded rectangles indicate the period during which the heater is on.


A
B
C
D
E
H
C
G
F
J
K
L
M

A.


A
B
C
D
E
G
F
H
K
L

B.


TEMPERATURE (°C)
0.9
0.6
0.3
0
-0.3
-0.6
TIME (minutes)

achieved by having a relatively constant heat sink and by activating a heat source in response to slight decreases in temperature from the set point. This is referred to as on-off control. In this cabinet, the cooling coils and fan function continuously, whereas the 500-watt heater is activated by a microswitch which responds to thermo-pressure changes in the sensing bulb of the Gallenkamp unit (see Fig. 2). These changes are communicated hydraulically (via capillary tube and bellows) to a temperature-compensated lever system which can be adjusted to a desired set point. The microswitch simultaneously activates a smaller heater around the capillary tube, providing negative feedback and reducing the oscillations which are typical of on-off temperature control. Fig. 3 illustrates the typical saw-tooth oscillations of the air temperature near the subject when its cloacal temperature is at 0°C, and both temperatures are monitored intermittently. Although the air temperature fluctuates about 1.2°C approximately every two minutes, these fluctuations are not detectable in the core temperature measured to within 0.1°C of a 1.4 gram skink. The oscillations of the air temperature are shorter in duration at higher set temperatures (approaching about one cycle per minute at 40°C), but have similar amplitudes over the entire operating range. At -5°C, the heater is activated very infrequently, indicating that the heat gained from outside the cabinet nearly balances the heat taken up by the cooling system.

All the thermistor and electrode leads were originally led through the panel at the back of the cabinet (see Fig. 2). The records of bioelectric activity (electroorganograms) taken under these conditions were particularly noisy due primarily to 50 cycle interference. Consequently, only the thermistor leads are plugged into the panel, whereas the electrode leads run between the door insulation to the preamplifiers.

EQUILIBRATION EXPERIMENTS

A series of tests indicated that it would be practicable to determine certain physiological responses of subjects in the cabinet over the range -5° to 40°C. To obtain minimum physiological rates, it was decided to equilibrate the subjects

for at least 30 minutes at each temperature station except 40°C (see below). The use of stations was to facilitate statistical treatment of the data. For convenience, these stations were at five degree intervals, but excluded 15°C and 25°C. Because the stations -5°C and 40°C are near to the lethal limits of the subjects, the physiological responses were determined separately for -5°C, 0° to 35°C and 40°C. It was found to be less stressful for the subjects and more convenient to begin equilibration experiments at 20°C. The subjects were warmed in steps to 35°C, allowed to cool to 0°C and then warmed in steps to 20°C again. There was no significant difference ($P > 0.5$) for acutely-measured cardiac rates obtained at 20°C at the beginning and end of experiments. For experiments at -5°C, the subjects were rapidly cooled to 0°C and then supercooled slowly. Since the skinks could only tolerate 40°C for 15-20 minutes, they were heated slowly to 35°C and rapidly to 40°C, then held at that temperature for as long as was necessary for the determination of the response.

APPENDIX D: CARDIO-ELECTROGRAPHY AND ELECTROORGANOGRAPHY

Electrography is the recording of changes of electrical potential obtained from the surface of an organ, hence cardio-electrography (Hoffman and Cranefield, 1960). Electroorganography is the recording of the superimposition of potential differences which are obtained from the surface of the body and associated with activities of organs and tissues (Morris, 1971). With appropriately-placed electrodes, electrical activity associated with cardiac, ventilatory and general muscular activity can be monitored. Records of these activities are termed electrocardiograms (ECG), electropneumograms (EPG) and electromyograms (EMG) respectively.

CARDIO-ELECTROGRAPHY

Open chest experiments were carried out on pithed skinks (L. zelandica) to ascertain the time relations and sequence of electrocardiac activity. Fine platinum wire (43 swg) was fashioned into spring electrodes which could be placed on to beating hearts without affecting the normal movement of the heart. Polarities of the recording equipment were arranged so that negative deflections were upward. Based on biophysical principles, when depolarisation occurs toward the exploring electrode, the main deflection will be positive. If repolarisation occurs in the same direction as depolarisation, the T wave will be negative. In one series of experiments, the exploring electrode was placed variously on the apex, the mid-ventral surface, and the base (near to the auriculo-ventricular junction) of the ventricle, and the indifferent electrode was in the pelvic musculature. As illustrated in Fig. 4 (a-c), depolarisation and repolarisation of the ventricle proceed from base-to-apex as demonstrated for other squamates by Mullen (1967) and Valentinuzzi et al. (1969b). When the exploring electrode is placed between the auricles on the dorsal surface, auricular depolarisation proceeds from the right to the left

Fig. 4: Electrograms.

a. Active electrode on apex of ventricle (25°C).

b. Active electrode on mid-ventral surface of ventricle (25°C).

c. Active electrode on base of ventricle (near AV junction) (25°C).

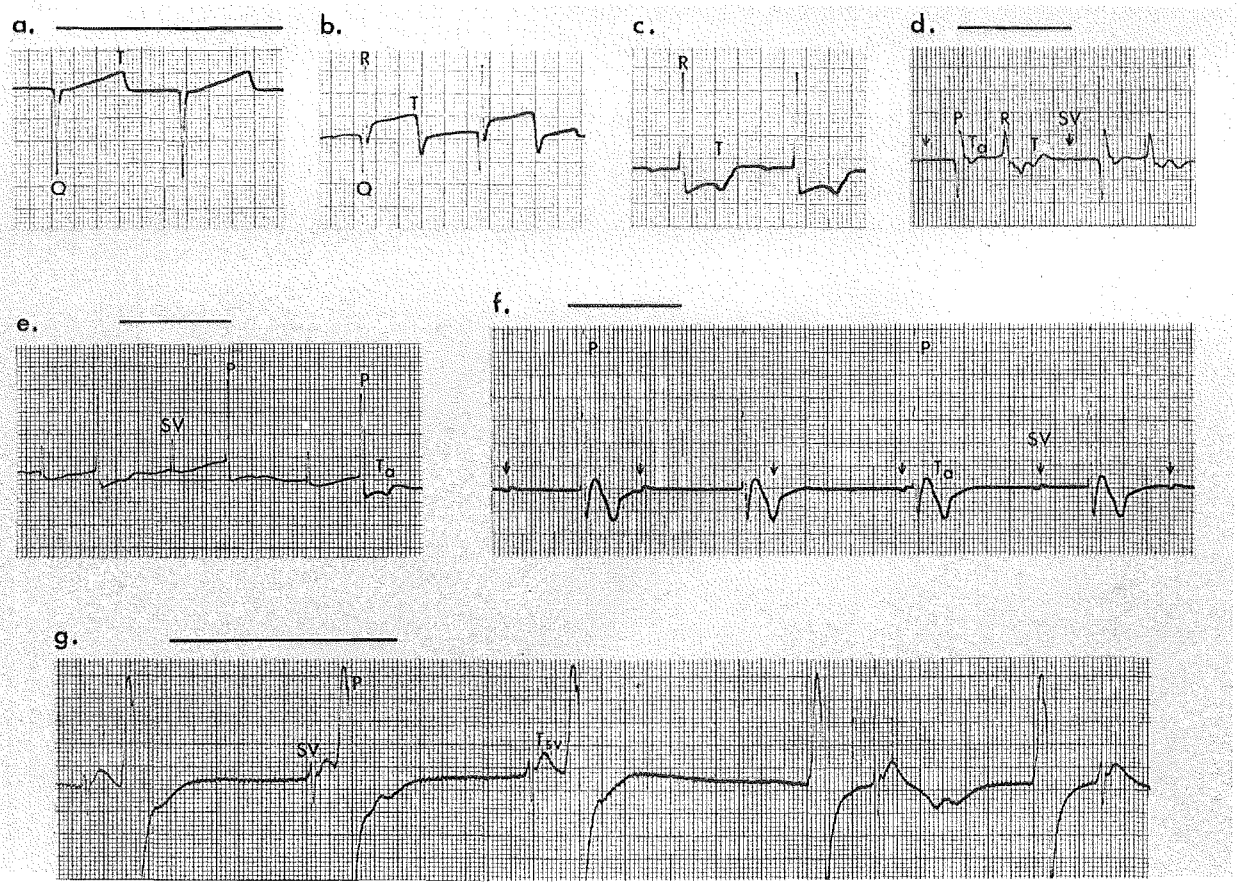
d. Heart tipped forward, active electrode is between the auricles and on the dorsal surface; first degree AV block and possibly first degree SA block; SV and T_a waves are evident (15°C).

e. Active electrode on sinus venosus (pacemaker) during third degree AV block (15°C).

f. Active electrode on right auricle. Apparent disassociation of SV and P waves; regular sinus rhythm (15°C).

g. Active electrode on sinus venosus; sinus rhythm irregular. T_{sv} is present (20°C).

Horizontal bars are 1 second, records a-c are at the same rate.


a.
T
Q
b.
R
T
Q
c.
R
T
d.
P
R
SV
Ta
T
e.
P
P
SV
Ta
f.
P
P
SV
Ta
g.
P
SV
T SV

auricle (see Fig. 4d). The act of tipping the heart forward to obtain this recording appears to have caused first degree AV and possibly first degree SA block. On the basis of many open chest experiments, it is apparent that the auriculo-ventricular (AV) junction and, to a lesser extent, the sinu-auricular (SA) junction are particularly sensitive to experimental manipulations. First degree (lengthened P-R int.), second degree (some P waves not followed by R waves, hence partial), and third degree (complete) AV block invariably occurred in this order. AV block does facilitate, however, the recording of sinus venosus and auricular electrograms. In these recordings, the T waves of the sinus venosus and auricles (T_{sv} and T_a respectively) can be detected (see Fig. 4, d-g). This has also been demonstrated by Valentinuzzi et al. (1969a) for snake hearts. In the SV electrogram shown in Fig. 4e, the durations of the intervals SV-T_{sv}, P-T_a, SV-P and SV-SV (equals P-P) are 288, 412, 440 and 1168 msec respectively (15°C). The SV-P interval seems rather long and may indicate first degree SA block in the preparation. The deflections labelled SV waves in the electrograms illustrated in this report were inscribed concurrent with the contraction of a portion of tissue on the posterior margin of the sinus venosus near to the SA junction. When a ligature is applied to the SA junction or when the respective tissues are cut apart, only the sinus tissue continues contracting, indicating that it is the pacemaker. In no case did the auricles and ventricle exhibit intrinsic automaticity. On the basis of these observations, it is not clear how SV and P waves can have different rhythms and appear disassociated from the normal sequence of activation (see Fig. 4, f and g). It is possible that some pacemaker activity may occur in the SA junction; this could be demonstrated by obtaining transmembrane potentials from this area. The existence of an SA node in reptiles has been histologically demonstrated (for a review of this literature see Chiodi and Bortolami, 1967) but as yet, electrophysiological evidence is lacking.

From the open chest experiments, several points were clarified regarding the relation of the electrical activity of the leiolopismid heart (cardio-electrograms) to electrical

activity obtained from the surface of the body (i.e. ECG). The signals from the sinus tissue (SV and T_{sv} waves) are too weak to be detected from the surface of the body. When ECG and electrograms are compared before and after AV block, the P-R seg. (see Fig. 7) is only slightly shorter than the duration of auricular repolarisation (P-T_a seg., which is measured from the end of the P wave to the end of the T_a wave). For this reason, the P-R seg. can be considered as an approximation of auricular repolarisation time. At 40°C, in fact, the P-R seg. and the P-T_a seg. are generally the same. Based on the vectors of cardiac depolarisation (see above), when the active electrode is placed anterior to the heart (as in the orthogonal YY lead used for ECG), the P wave should be largely positive and ventricular depolarisation should consist of negative deflection (R wave). No Q wave would be expected from this lead and an S wave would be largely artefactual, i.e. due to capacity-coupling and electrical differentiation described in detail below. The duration from the beginning of the R wave to the nadir of the S wave was found to correspond to the duration of the Q wave obtained from the apex of the ventricle.

ELECTROORGANOGRAPHY

Leads and electrodes

To record cardiac and ventilatory responses of small lizards at different temperatures, it was desirable to employ a lead with electrodes which could be applied easily, which produced relatively large analysable waveforms including the smaller P and T waves, and which were unstressful to unanaesthetised subjects even when implanted for hours. An orthogonal YY or longitudinal lead, consisting of a jugular and a cloacal electrode proved satisfactory. The jugular electrode was implanted subcutaneously about mid-way between the ear opening and the forelimb, and the other electrode was placed into the cloaca (see Fig. 6). A comparable lead was used by Valentinuzzi et al. (1969b) for snakes, which skinks resemble in terms of anatomical configuration. By autopsy, the jugular electrode was found to lie antero-ventral to the heart.

Fig. 5: Experimental subject with electrodes and leads.

Letters refer to the following:

A. Metal pan (only a portion of which is shown) in which subjects were placed.

B. Thermistor lead.

C. Cloacal electrode (also shown in detail at the right).

D. Jugular electrode (as in C).

E. Subject taped on to balsa wood stretcher with electrodes and leads in place.

Fig. 6: Block diagram of experimental apparatus for electroorganography.

Letters refer to the following:

A. As in Fig. 5, but showing two subjects each fitted with leads.

B. Thermistor leads to panel on temperature cabinet.

F. Temperature cabinet (see Appendix C for details)

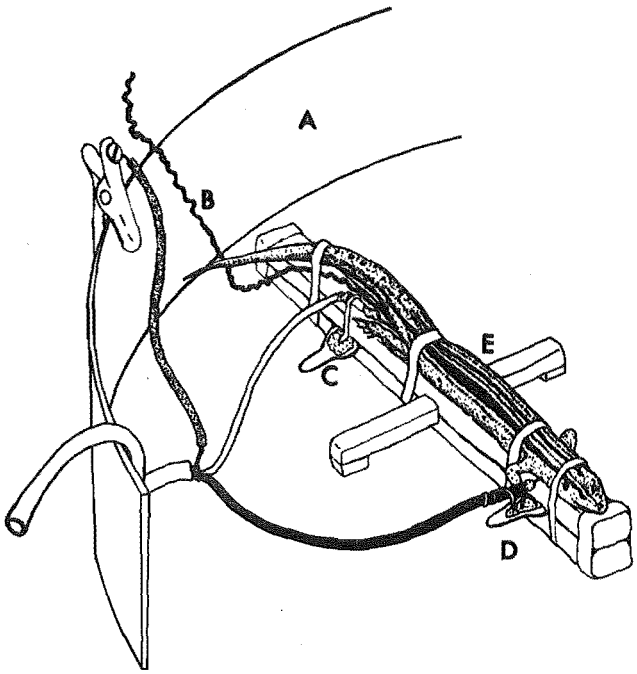
G. Modified Wheatstone bridge with helipot and microammeter.

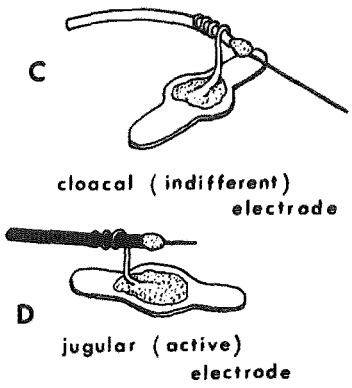
H. Tektronix AC Preamplifiers (Model 122).

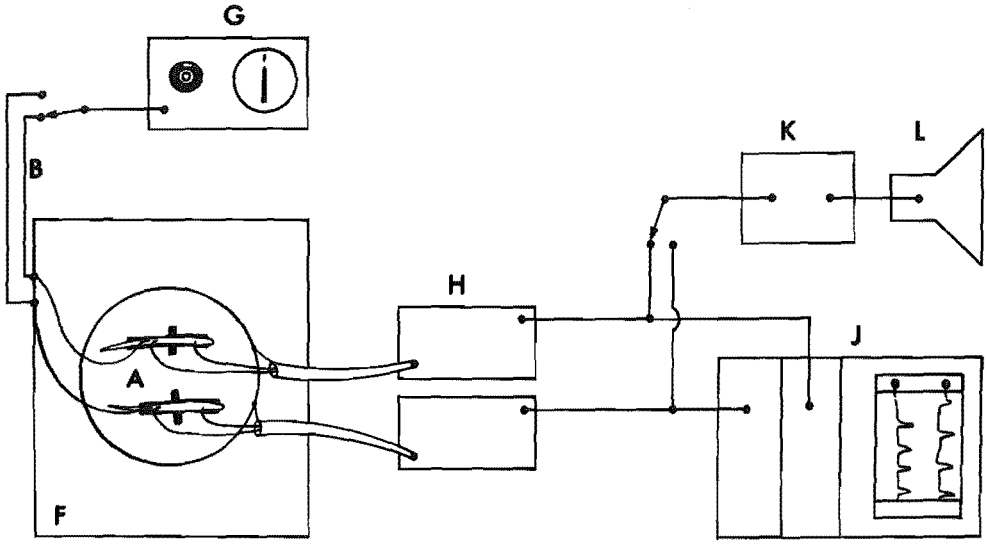
J. Hewlett-Packard DC Preamplifiers (Model 350-2700C) and Paper Recording System (Model 7712B).

K. Sound amplifier.

L. Speaker.


A
B
C
D
E


C
cloacal (indifferent)
electrode
D
jugular (active)
electrode


G
B
K
L
H
J
A
F

Platinum electrodes (28 swg) were soldered to separate wires of a shielded cable. The shield was connected to the metal pan containing the subjects and was grounded to the electronic equipment (see Figs 5 and 6). The electrodes were attached to metal tags (shown in detail in Fig. 5) which could be pushed into the gap between the two balsa wood boards that comprised the long axis of the cross-shaped stretchers.

General procedure and instrumentation

The subjects were taped to the stretchers and fitted with the electrodes and a bead-type thermistor (also in the cloaca). The prepared subjects were placed into a metal pan to facilitate their transfer to the temperature cabinet (see Appendix C) and to shield them from electrical interference. The body temperatures were monitored by a modified Wheatstone bridge consisting of a series of fixed resistors, a helipot and a microammeter. This device was constructed in the Zoology Department. The bioelectric signals were fed push-pull into preamplifiers and then into either a sound amplifying or paper recording system (see Fig. 6).

As a consequence of the electrodes and instrumentation used in this study, the electrocardiac signals were electrically differentiated. Because of the capacitive nature of the interface impedance between metal electrodes and the tissue electrolyte, a time constant is introduced into the circuit resulting in the loss of low frequency response. The only conventional metal electrode with a better low frequency response than platinum, is the Ag:AgCl electrode (Cooper, 1963). This electrode is, however, not as robust and cannot be reused as easily as a platinum electrode. Distortions of the signals can be minimised by using electrodes of relatively large surface area and by connecting them to an amplifier with a high input impedance (Geddes, 1972). Both the active and indifferent electrodes used in this study were relatively long and were fashioned from platinum wire 0.45 mm in diameter. The active electrode had an effective area of about 15 mm^2. The thickness of the wire did not appear to be stressful when placed subcutaneously, ventral to the sternum. With the electrodes and leads used in this study, RS potentials between

500 and 800 μV in amplitude were measured at temperatures above 20°C. The amplitudes decreased to below 100 μV during acute supercooling to -5°C. During chronic exposures to this temperature, however, the RS potentials dropped gradually in amplitude until, after about ten hours, no potentials could be detected from the surface of the body. When these subjects were warmed to 20°C, the RS potentials reappeared as about 10-25 μV in amplitude at about -1°C, increasing rapidly until they were within the normal range of RS amplitudes at 20°C.

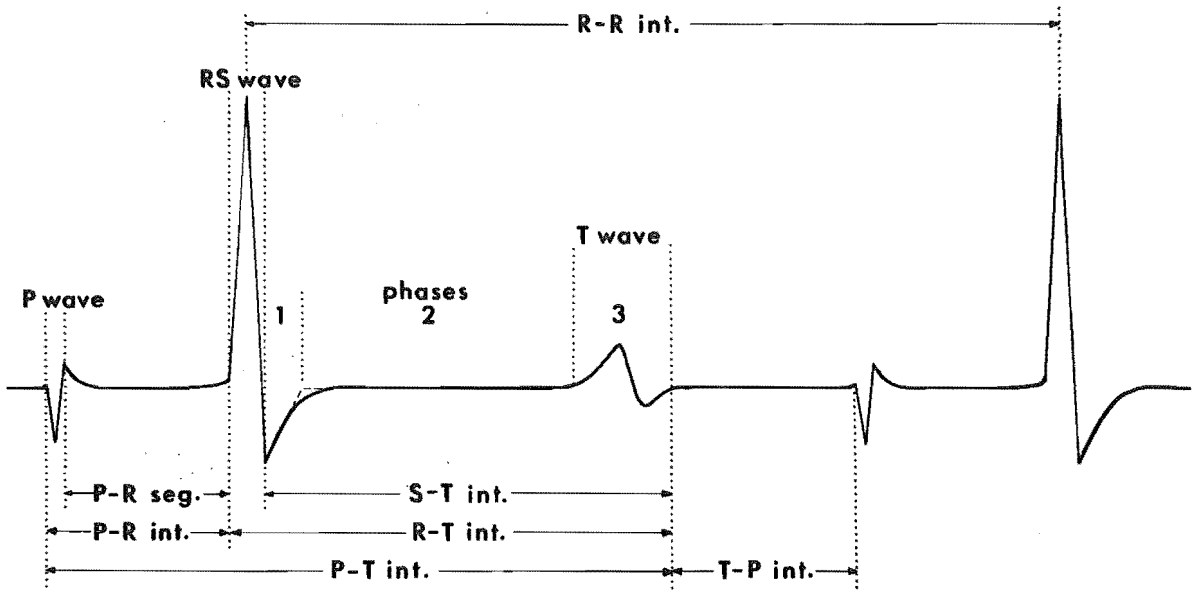
Another source of electrical differentiation is AC preamplification. By feeding sinusoidal waves of known voltage and frequency into the experimental arrangement used in this study (see Fig. 6), it was determined that with the low frequency cut-offs employed, only the amplitudes and not the durations of the waves (wavelengths) are affected. The amplitudes of the ECG signals were not utilised in the detailed analysis of the ECG. When the lowest frequency response (0.2 hertz) was used to record the ECG, the waveforms were identical to those obtained by Valentinuzzi et al. (1969b) for snakes. They employed a comparable lead with stainless steel electrodes and a DC amplifier. It was usually necessary in the present study to use the next lowest setting (0.8 hertz) to reduce the baseline drift. This practice was the source of most of the electrical differentiation (see Caceres, 1973). Of the three major waveforms of the ECG, the R wave was the most affected. As a result, the R wave was followed by an undershoot (an artefactual S wave) and what resembled an RC-type decay to the baseline.

Recording and interpretation of EOG events

To obtain ventilatory and cardiac rates, records were taken at low paper speeds, generally 2.5 mm/sec. When the EPG activity was obscured by ECG activity above 20°C, the high frequency signals associated with ventilatory activity were transduced into sounds which could be counted. To obtain more detailed information regarding the time relations of EOG events, paper speeds up to 50 mm/sec were used. By the use of vernier calipers and a stereo microscope with a calibrated eye-piece, ECG and EPG events could theoretically be measured to

Fig. 7: Diagrammatic representation of an ECG.

The nomenclature and measurement of major events, including waves, intervals (int.) and a segment (seg.), are illustrated. The interpretation of events is described in the text. The waves are shown as electrically differentiated which is typical of the ECG obtained in this study.


R-R int.
RS wave
T wave
P wave
phases
1
2
3
P-R seg.
S-T int.
P-R int.
R-T int.
P-T int.
T-P int.

plus or minus 1 msec. In fact, the accuracy of the measurements depended on the clarity of the waveforms and particularly their beginnings and ends. Data from interpretable records only were included in the analyses.

Ventilatory movements at low temperatures are sufficiently slow to allow electrical (EPG) and corresponding mechanical events to be readily determined. The components of the ventilatory cycle were found to be comparable to those described by Templeton and Dawson (1963) for Crotaphytus collaris. The electropneumic cycle is characterised by periods of electrical inactivity (apnoea) followed by two bursts of electrical activity which are separated by a discrete period of electrical quiescence. These bursts of electrical activity are associated with active expiration and active inspiration. No electrical signals are detected during either the short period of passive inspiration, or the final, partial expiratory movement, both of which are due to the elastic recoil of the ribs. The EPG signals are composed of higher frequency components than occur in ECG, although at temperatures above 30°C there is some overlap in frequencies. In addition, the amplitude of EPG signals does not increase as markedly with temperature as does the amplitude of ECG signals (particularly the R wave). For these reasons, it was necessary to attenuate the EOG signals considerably to record EOG activities at higher temperatures. Even by filtering out a large portion of the ECG, however, it was not possible with the equipment available to obtain satisfactory EPG above about 20°C. There was also the problem that the duration of the electropneumic cycle is similar to the duration of the R-R int. at temperatures above 20°C. The electrically-quiescent period was often difficult to measure at 20°C due to interference from ECG activity and due to its brevity.

The ECG events measured in this study are illustrated in Fig. 7. The P wave is the deflection produced by auricular depolarisation. The P-R seg., as explained above, is an approximation of the duration of auricular repolarisation. It is also a measure of the AV conduction time. The P-R int. includes most of the period of auricular electrical activity. The RS wave results from ventricular depolarisation. The total

duration of the undifferentiated R wave should correspond to the duration of ventricular depolarisation, the reversal of polarisation and phase 1 of the recovery (repolarisation) of the ventricular action potential or transmembrane potential (TMP) (see Hecht, 1957; Hoffman and Cranefield, 1960; Tricoche, 1968). The reversal of polarisation apparently coincides with the nadir of the S wave (see above). Phase 1 would then correspond to the period between the nadir and the end of the S wave. Because of the difficulty of determining the precise end-point of the S wave, it was measured as illustrated in Fig. 7. Phase 2 ("plateau") of the recovery of the TMP corresponds to the S-T seg. and phase 3 occurs simultaneously with the T wave (see Fig. 7). The S-T int. includes the three phases of ventricular repolarisation. The R-T int. represents the total period of ventricular electrical activity. The relationship between the auricular TMP and the corresponding ECG is similar (see Hoffman and Cranefield, 1960), although the difficulty of recording the T_a from the body surface precludes a comparable analysis for the auricle. The P-T int. includes the P-R int. and the R-T int. and represents the total electrical activity of the auricles and ventricles. The T-P int. is the period during which these tissues are inactive. The R-R int. is easier to measure than the P-P int. and would normally be identical with it.

APPENDIX E: MUSCLE TENSION APPARATUS

To measure isometric twitch tension development of small muscles *in vitro* over a wide range of temperatures, an apparatus was constructed as illustrated in Fig. 8. The muscle preparation was mounted vertically on to the jig by fine steel wires which were tied on to the ends of the preparation. These wires were attached to the leads of the stimulating electrodes. The anodal electrode ran down the aerating tube as an insulated wire and had a hook at the end. The cathodal electrode was soldered to a fine wire which coiled up to join a thread from the mechano-electrical transducer tube (RCA 5734) and was led down through a hole in the stopper to provide the other attachment for the preparation. The transducer could be moved in two planes relative to the jig for fine adjustment of resting tension. The jig and transducer, could also be raised and lowered as a single unit (see Fig. 8, double-headed arrow) by a Palmer screw stand. In this way, the apparatus could be moved between two baths without disturbing the position or tension of the preparation on the jig. The test bath could be maintained at set temperatures over the range 0° to $45^{\circ}C$, whereas the reference bath was held at $20^{\circ}C$. Both baths were regulated to within $0.1^{\circ}C$. The circuitry for the mechano-electrical transducer tube was as described by Geddes and Baker (1968). The output from the transducer was fed into a Hewlett-Packard DC Preamplifier (Model 350-2700C) and Paper Recorder (Model 7712B). Stimulation was from a Grass Stimulator (Model S4G) through a Stimulus Isolation Unit (Model SIU-4B). Oxygen was bubbled continuously through the Ringer's solution. This solution was similar to that of Licht (1964) and consisted of the following: 155 mM NaCl, 4 mM KCl, 2 mM $CaCl_2$, 1 mM $MgSO_4$, 5.6 mM glucose and 2mM Sørensen's phosphate buffer at a pH of 7.2 (glass electrode). The experimental procedures were similar to those used by previous workers except that the preparation was equilibrated for three rather than five minutes (see Licht, 1964). Isometric muscle twitches obtained by widely-spaced pulses were recorded at

Fig. 8: Diagram of apparatus for measuring muscle tension at test and reference temperatures.

Letters in the diagram refer to the following:

A. Palmer rackwork blocks connected to other Palmer fittings

B. RCA 5734 mounted in an aluminium clamp and insulated from the rest of the apparatus by a Tufnol insulator

C. Jig consisting of an aerating tube mounted in a perspex stopper

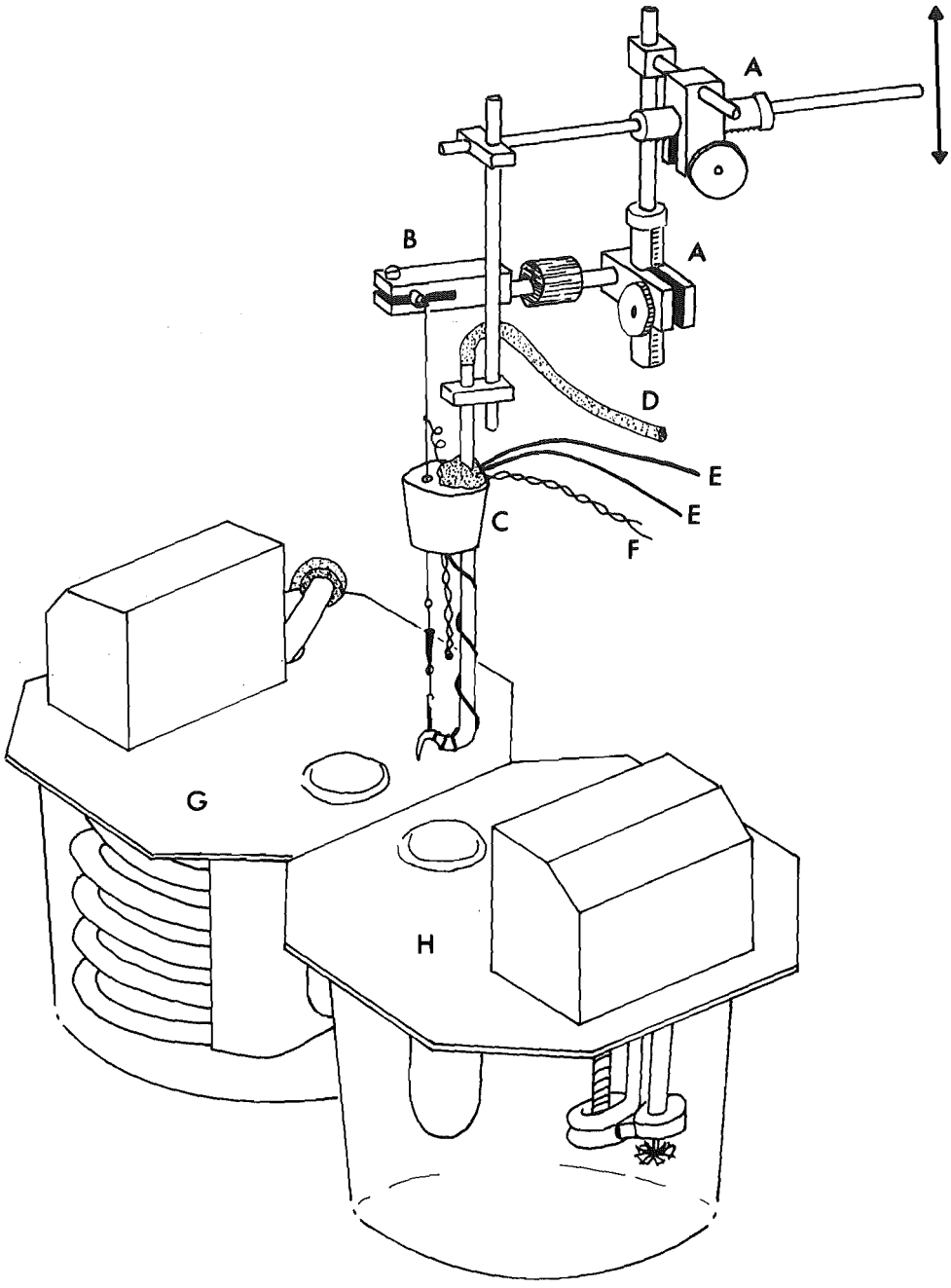
D. Oxygen supply

E. Leads of stimulating electrodes which run to both ends of muscle preparation and which are held in place on the stopper with plasticine

F. Lead to bead-type thermistor which is adjacent to muscle

G. Test bath (methylated spirits) with a cooling coil and Tempunit for temperature control and with a tube containing Ringer's solution

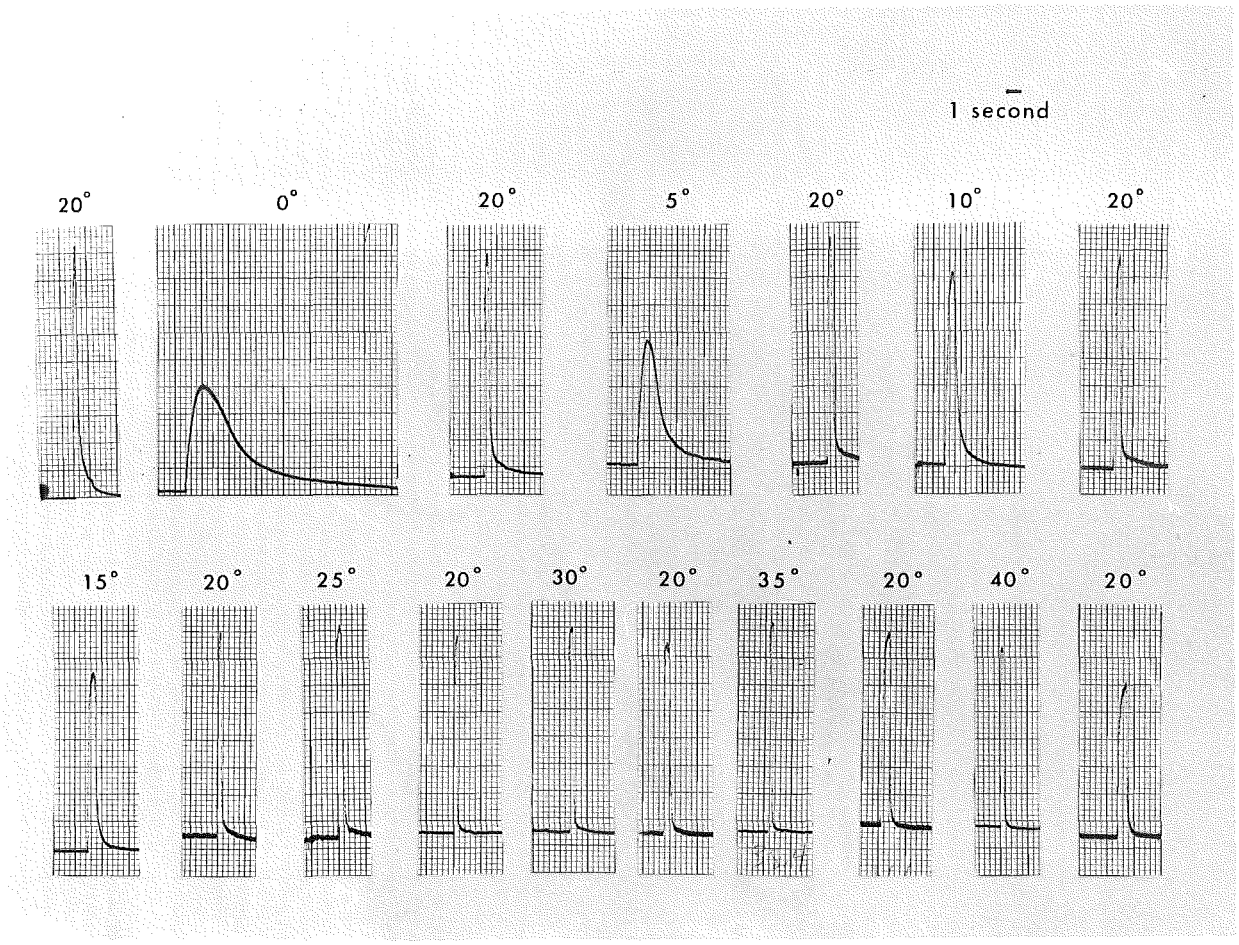
H. Reference water bath ($20^{\circ}C$) with Tempunit and a tube (as in G).


A
B
A
D
E
E
C
F
G
H

test temperatures at five degree intervals and at the reference temperature between each test temperature. Fig. 9 illustrates the appearance of the twitches for one preparation over the range 0^{o} to $40^{o}C$.

Fig. 9: Mechanograms

These records illustrate isometric twitches from one preparation produced by supramaximal stimulation. Preparation was tested alternatively at the reference temperature ($20^{o}C$) and at a test temperature (0^{o} to $40^{o}C$).


1 second
20°
0°
20°
5°
20°
10°
20°
15°
20°
25°
20°
30°
20°
35°
20°
40°
20°

APPENDIX F: TABULATED RESULTS

These results include more detailed statistics of data which are presented graphically in Section 1. The format has been standardised to include the temperature ($T^{o}C$), number of individuals (n), mean value ($\overline{Y}$), range, standard deviation (S), standard error of the mean ($S_{\overline{Y}}$), and t-values when appropriate.

Table 1: Oxygen consumption - whole animal

Y values are expressed as µl oxygen/g initial body weight/hr at STP ($0^{o}C$ and 760 mm of mercury). Summer and winter values have been lumped, t-values are from comparison of these seasonal values at the different temperatures.

$T^{o}C$	n	$\overline{Y}$	range	S	$S_{\overline{Y}}$	t-values
-5	15	5.85	2.9-9.0	1.71	0.44	-
0	25	12.96	9.2-17.3	2.22	0.44	0.167
5	57	25.79	18.3-37.8	4.75	0.63	0.333
10	45	50.43	37.0-72.5	8.53	1.64	0.143
20	47	152.9	100-207	26.6	3.9	0.796
30	40	329.9	254-472	56.3	8.9	0.661
35	51	508.7	409-621	76.1	10.7	1.379

Table 2: Ventilatory rate

Y values are expressed as breaths/minute.

$T^{o}C$	n	$\overline{Y}$	range	S	$S_{\overline{Y}}$
0	-	-	0.1-6.1	-	-
5	21	7.41	4.5-11.1	1.82	0.40
10	23	12.56	8.4-20.0	3.35	0.70
20	24	21.01	7.1-39.0	8.45	1.73
30	20	42.93	21-70	13.49	3.02
35	20	47.92	29-76	13.30	2.97
40	10	77.50	68-92	6.84	2.16

Table 3: Electropneumographic events

Y values are durations, expressed in milliseconds. For convenience, the events are expressed as follows: Total period of electrical activity (Total), active expiration (A), electrically quiescent period (B) and active inspiration (C).

	event	n	$\bar{Y}$	range	S	$S_{\bar{Y}}$
0°C	Total	3	2534	2045-3080	425	245
	A	3	914	637-1262	260	150
	B	3	281	240-325	35	20
	C	3	1362	1130-1648	215	124
5°C	Total	13	1356.9	1148-1689	204.7	56.8
	A	13	516.8	318-709	115.7	32.1
	B	13	222.5	113-400	79.0	21.9
	C	13	629.2	450-832	108.0	30.0
10°C	Total	14	798.6	491-1060	181.5	56.8
	A	8	371.0	178-469	94.5	32.1
	B	8	122.0	63-221	45.4	21.9
	C	8	366.5	227-589	105.6	30.0
20°C	Total	12	591.0	379-1032	205.0	59.1
	A	2	198.5	165,232	-	-
	B	2	56.0	53,59	-	-
	C	2	218.5	217,220	-	-

Table 4: Cardiac rate

Y values are expressed as beats/minute.

T°C	n	$\bar{Y}$	range	S	$S_{\bar{Y}}$
-5	16	0.62	0.30-0.81	0.14	0.04
0	30	4.62	3.3-6.1	0.81	0.15
5	31	12.96	10.1-16.2	1.48	0.27
10	30	24.15	18.9-29.6	2.77	0.51
20*	31	62.54	45.8-78.0	8.18	1.47
30	27	122.07	99.7-144.7	11.13	2.14
35	25	165.29	149.0-189.5	11.55	2.31
40	16	200.69	173.5-238.0	18.38	4.59

* When values for 20°C obtained at beginning and end of an experiment are compared (n = 16), the t-value is 0.241.

Table 5: Electrocardiographic events

Y values are durations, expressed in milliseconds. The description and interpretation of ECG events are presented in detail in Appendix D.

	event	n	$\overline{Y}$	range	S	$S_{\overline{Y}}$
-5°C	P wave	2	230	220,240	-	-
	P-R seg.	2	2740	2360,3120	-	-
	P-R int.	7	2994	2560-3860	439	53
	RS wave	3	451	340-532	-	-
	S-T int.	3	5484	4860-6280	-	-
	R-T int.	6	6172	5200-6840	-	-
	P-T int.	5	8946	8400-9400	555	229
	T-P int.	5	137,764	80,560-188,880	44,286	19,800
	R-R int.	7	143,908	89,000-197,960	38,219	14,430
0°C	P wave	8	120.0	86-144	20.2	7.2
	P-R seg.	8	1379.5	1070-1676	200.9	71.0
	P-R int.	23	1655.9	1158-2240	287.3	59.9
	RS wave	19	186.1	140-232	25.4	5.8
	S-T int.	14	3518.6	2308-4080	526.6	141.3
	R-T int.	18	3619.6	2440-4320	560.5	132.1
	P-T int.	18	5355.6	4048-6508	696.3	164.1
	T-P int.	17	7527.0	3733-11,600	2133.6	517.5
	R-R int.	25	13,108.0	9890-18,130	2375.0	475.0
5°C	P wave	10	57.0	48-70	6.6	2.1
	P-R seg.	10	878.2	736-1004	107.7	34.1
	P-R int.	24	981.5	800-1284	107.6	22.0
	RS wave	16	102.6	84-146	16.0	4.0
	S-T int.	14	1759.1	1276-1988	188.8	50.5
	R-T int.	17	1910.0	1422-2288	198.3	48.1
	P-T int.	17	2860.5	2258-3328	245.8	59.6
	T-P int.	17	1738.7	920-3422	529.2	128.4
	R-R int.	24	4613.8	3700-6280	569.7	116.3

	event	n	$\bar{Y}$	range	S	$S_{\bar{Y}}$
10°C	P wave	12	33.6	28-42	5.1	1.2
	P-R seg.	12	541.2	459-670	57.9	16.7
	P-R int.	24	589.1	501-746	69.2	14.1
	RS wave	21	53.2	40-60	5.2	1.1
	S-T int.	18	1219.7	984-1585	142.0	33.5
	R-T int.	21	1280.0	1040-1632	152.0	33.3
	P-T int.	21	1845.3	1566-2336	187.5	40.9
	T-P int.	21	656.5	306-1198	229.3	50.0
	R-R int.	24	2585.5	2018-3560	395.7	80.8
20°C	P wave	16	21.4	18-24	2.0	0.5
	P-R seg.	16	228.8	197-308	28.5	7.1
	P-R int.	27	251.2	186-330	31.7	6.1
	RS wave	26	34.7	24-44	4.3	0.8
	S-T int.	26	594.2	465-740	76.4	15.0
	R-T int.	27	628.1	497-792	74.6	14.4
	P-T int.	27	131.3	40-277	60.1	11.6
	T-P int.	27	876.1	713-1119	91.8	17.7
	R-R int.	27	1009.4	752-1288	138.8	26.7
30°C	P wave	16	17.4	15-24	2.1	0.5
	P-R seg.	16	106.6	88-136	14.6	3.7
	P-R int.	25	125.6	103-156	13.7	2.7
	RS wave	21	30.1	25-37	3.5	0.8
	S-T int.	20	297.8	229-368	34.7	7.8
	R-T int.	22	331.0	260-402	35.0	7.5
	P-T int.	22	453.9	377-542	38.9	8.3
	T-P int.*	22	45.2	-3 - 104	30.8	6.6
	R-R int.	25	503.8	377-606	53.1	10.6

* A negative number indicates some overlap of the P and T waves. Means and statistical data were obtained by treating small negative numbers as zero.

	event	n	$\bar{Y}$	range	S	$S_{\bar{Y}}$
35°C	P wave	18	15.6	12-20	2.3	0.5
	P-R seg.	18	77.8	68-93	8.5	2.0
	P-R int.	22	92.8	80-110	8.4	1.8
	RS wave	20	26.9	21-35	4.2	0.9
	S-T int.	20	210.9	164-257	21.3	4.8
	R-T int.	20	238.5	192-296	23.6	5.3
	P-T int.	20	329.3	282-396	26.8	6.0
	T-P int.*	20	29.1	-2 - 70	18.5	4.1
	R-R int.	22	362.3	289-446	38.8	8.3

* See note for 30°C.

40°C	P wave	15	12.1	10-14	1.4	0.4
	P-R seg.	15	64.1	52-75	6.7	1.7
	P-R int.	17	76.8	65-88	6.3	1.5
	RS wave	17	21.5	18-24	1.9	0.5
	S-T int.	17	157.7	126-204	19.7	4.8
	R-T int.	17	179.2	149-228	20.3	4.9
	P-T int.	17	255.9	221-300	21.8	5.3
	T-P int.*	15	47.1	9-75	16.1	4.2
	R-R int.	15	298.9	251-346	26.8	6.9

* Two individuals had SA block so were not included in these samples. Except for the unusually long T-P int. (hence, long R-R int.), the intervals were similar to records without SA block.

Table 6: Relative values of ECG events

Each entry represents the mean ratio, sample size (n) and range of ratios.

Ratio	Temperature °C							
	-5	0	5	10	20	30	35	40
P wave/P-R int.	0.079(2) 0.066,0.092	0.080(8) 0.052-0.095	0.062(10) 0.050-0.084	0.060(12) 0.050-0.084	0.087(16) 0.067-0.110	0.141(16) 0.114-0.182	0.167(18) 0.130-0.209	0.161(15) 0.121-0.203
RS wave/R-T int.	0.074(3) 0.065-0.085	0.052(18) 0.039-0.087	0.054(16) 0.043-0.080	0.042(21) 0.029-0.054	0.057(26) 0.038-0.083	0.092(21) 0.076-0.119	0.114(20) 0.074-0.146	0.115(17) 0.096-0.136
*P-R int./P-T int.	0.320(5) 0.272-0.392	0.315(18) 0.250-0.397	0.323(17) 0.251-0.368	0.307(21) 0.237-0.359	0.289(27) 0.234-0.348	0.291(22) 0.227-0.328	0.279(20) 0.242-0.341	0.303(17) 0.240-0.336
P-R int./R-R int.	0.023(7) 0.014-0.038	0.131(23) 0.083-0.198	0.214(23) 0.153-0.276	0.231(24) 0.163-0.324	0.256(27) 0.190-0.337	0.250(25) 0.197-0.310	0.257(22) 0.221-0.341	0.254(15) 0.210-0.318
R-T int./R-R int.	0.045(6) 0.030-0.068	0.293(18) 0.229-0.372	0.419(17) 0.302-0.489	0.514(21) 0.405-0.604	0.624(27) 0.536-0.698	0.665(22) 0.541-0.730	0.667(20) 0.578-0.751	0.594(15) 0.537-0.666
*P-T int./R-R int.	0.068(5) 0.044-0.096	0.423(18) 0.329-0.569	0.630(17) 0.455-0.724	0.749(21) 0.628-0.858	0.880(27) 0.776-0.970	0.915(22) 0.802-1.005	0.924(20) 0.836-1.006	0.848(15) 0.775-0.963

* Since sample sizes are the same, (R-T int./P-T int.) = 1 - (P-R int./P-T int.)
(T-P int./R-R int.) = 1 - (P-T int./R-R int.)

Table 7: Oxygen consumption - Tissues

Y values are expressed in µl oxygen/g wet weight/hr.

	$T^{o}C$	n	$\bar{Y}$	range	S	$S_{\bar{Y}}$
Intestine	5	5	56.12	52.1-59.4	2.79	1.25
	10	5	84.62	79.4-96.0	5.91	2.64
	20	10	252.1	200-316	32.5	10.3
	30	11	510.4	385-629	71.3	21.5
	35	8	684.8	590-810	66.8	23.6
	40	9	756.8	613-897	82.1	27.4
Kidney	5	4	52.75	42.5-58.1	6.04	3.02
	10	4	83.73	66.6-97.8	13.48	6.74
	20	7	232.6	196-261	20.5	7.8
	30	8	426.5	331-503	64.8	22.9
	35	6	555.7	489-680	75.1	30.7
	40	5	698.4	555-839	106.7	44.7
Liver	5	5	31.74	29.3-33.8	1.44	0.64
	10	5	41.68	40.3-46.1	2.22	0.99
	20	12	95.67	74-118	14.48	4.18
	30	11	178.9	137-223	26.3	7.9
	35	8	243.3	188-288	34.3	12.1
	40	9	312.3	235-363	43.4	14.5
Brain	5	4	59.73	56.1-61.3	21.13	1.06
	10	3	85.17	83.4-86.1	1.25	0.72
	20	10	180.7	152-199	13.8	4.4
	30	10	407.4	368-452	28.0	8.8
	35	7	604.6	580-635	21.5	8.1
	40	8	904.4	754-1030	92.8	32.8

	$T^{o}C$	n	$\bar{Y}$	range	S	$S_{\bar{Y}}$
Fat Body	5	5	6.54	3.0-8.9	2.81	1.26
	10	5	8.86	7.8-10.4	0.85	0.38
	20	8	21.19	18.1-23.7	2.80	0.99
	30	8	33.95	24.4-50.6	7.20	2.55
	35	5	47.92	34.2-64.4	10.90	4.88
	40	6	55.87	42.8-96.0	18.39	7.51
Heart	5	4	62.53	51.6-80	10.68	5.34
	10	5	105.3	84-130	15.6	6.97
	20	9	209.1	185-232	16.1	5.4
	30	8	278.6	242-327	28.4	10.1
	35	7	401.6	319-527	64.6	24.4
	40	7	589.3	450-706	85.0	32.1
Skeletal Muscle	5	4	12.75	9.0-16.2	2.59	1.29
	10	5	13.90	10.0-17.5	2.52	1.13
	20	11	30.56	16.0-56.6	13.27	4.00
	30	9	38.16	24.8-62.8	13.40	4.47
	35	6	41.68	26.4-54.8	11.68	4.77
	40	6	45.45	31.2-78.6	16.46	6.72
Testis	5	4	25.65	23.8-29.9	2.47	1.24
	10	4	35.83	32.4-37.4	2.03	1.02
	20	8	117.2	105-130	7.3	2.6
	30	8	286.8	215-334	35.3	12.5
	35	7	430.4	353-520	49.4	18.7
	40	6	564.5	526-620	32.5	13.3

Table 8: Isometric twitch tension development *in vitro* of the puboischiotibialis muscle.

Y values are expressed as per cent of maximum tension.

$T^{\circ}C$	n	$\overline{Y}$	range	S	$S_{\overline{Y}}$
0	12	50.82	41.0-61.1	6.72	1.94
5	12	70.25	57.0-80.1	6.53	1.89
10	12	75.80	70.1-81.6	3.61	1.04
15	12	82.95	74.7-93.2	4.72	1.36
20	12	89.99	85.0-95.0	2.95	0.85
25	12	92.42	76.8-100.0	7.63	2.20
30	12	95.28	87.8-100.0	5.36	1.55
35	12	93.79	77.0-100.0	7.33	2.12
40	12	69.28	54.0-85.3	9.95	4.53
45	3	32.53	26.5-37.4	4.53	2.61

LITERATURE CITED

Caceres, C.A. 1973. Electrical activity of the heart. In Engineering principles of biology (Brown, J.H.U. and Gann, D.S. eds) Vol. II. Academic Press, New York.

Chiodi, V. and Bortolami, R. 1967. The conducting system of the vertebrate heart. Edizioni Calderini, Bologna.

Cooper, R. 1963. Electrodes. Amer. J. EEG Technol., 3: 91-101.

Geddes, L.A. 1972. Electrodes and the measurement of bioelectric events. Wiley-Interscience, New York.

Geddes, L.A. and Baker, L.E. 1968. Principles of applied biomedical instrumentation. J. Wiley and Sons, New York.

Hoffman, B.F. and Cranefield, P.F. 1960. Electrophysiology of the heart. McGraw-Hill, New York.

Licht, P. 1964. A comparative study of the thermal dependence of contractility in saurian skeletal muscle. Comp. Biochem. Physiol., 13: 27-34.

Morris, R.W. 1971. On some aspects of the biology, thermal relations and thermophysiology of Leiolopisma lineoocellatum. Unpublished M.Sc. thesis, University of Canterbury, New Zealand.

Mullen, R.K. 1967. Comparative electrocardiography of the squamata. Physiol. Zool., 40: 114-126.

Templeton, J.R. and Dawson, W.R. 1963. Respiration in the lizard Crotaphytus collaris. Physiol. Zool., 36: 104-121.

Tricoche, R. 1968. Electrophysiologie cardiaque comparee dans la serie animale. Annales Univ. Abidjan, Serie E: Ecologie, 1: 255-349.

Umbreit, W.W., Burris, R.H. and Stauffer, J.F. 1972. Manometric and biochemical techniques. Burgess Publishing Co., Minneapolis.

Valentinuzzi, M.E., Hoff, H.E. and Geddes, L.A. 1969a. Observations on the electrical activity of the snake heart. J. Electrocardiology, 2: 39-50.

Valentinuzzi, M.E., Hoff, H.E. and Geddes, L.A. 1969b. Electrocardiogram of the snake: Effect of the location of the electrodes and cardiac vectors. J. Electrocardiology, 2: 245-252.